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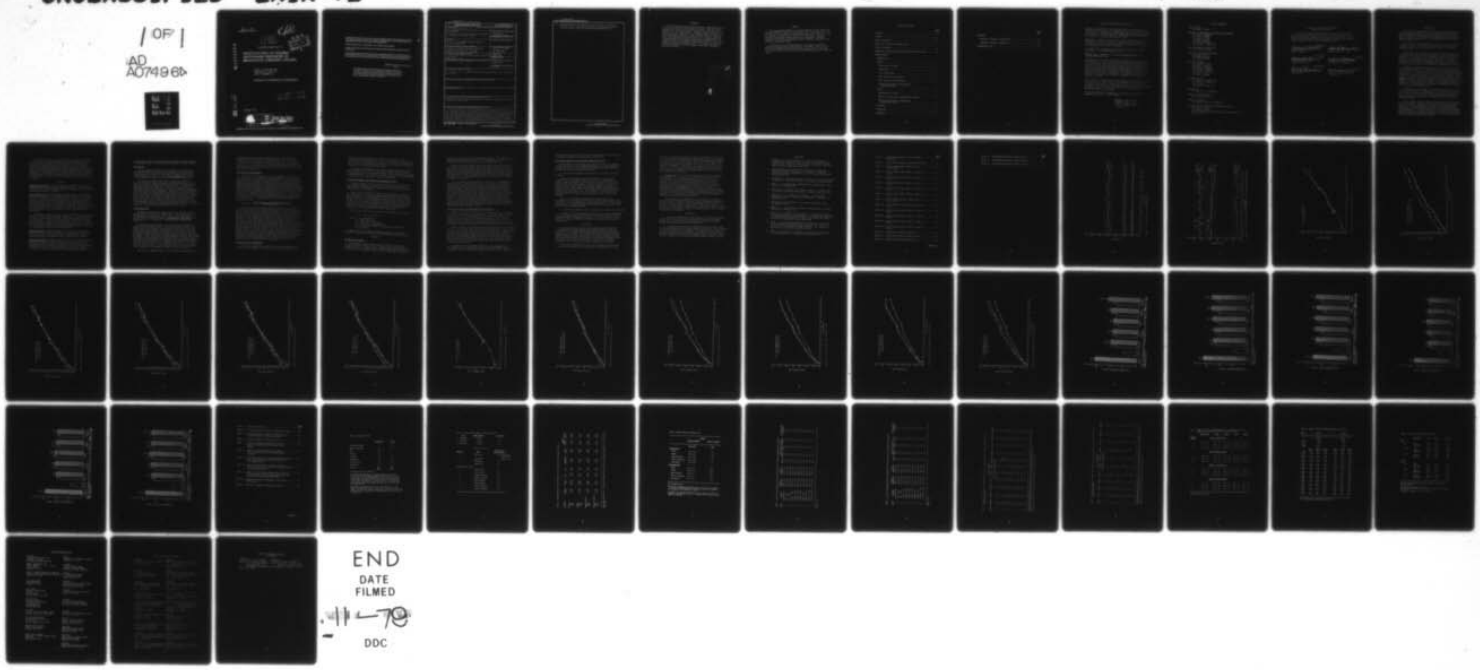

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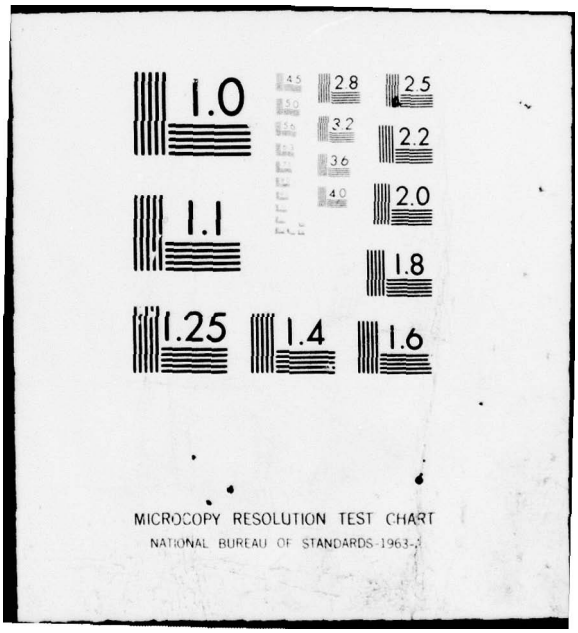
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INVESTIGATION OF POSSIBLE ANTITHIAMIN PROPERTIES IN IRRADIATION STERILIZED CHICKEN

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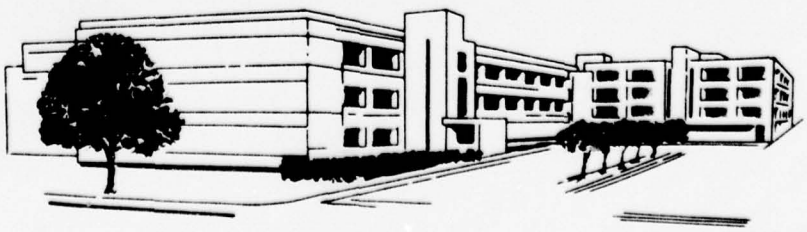
AUGUST 1979



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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Comittee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

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Irradiated Chicken, Antithiamin, antivitamin, thiamin, transketolase, thiamin pyrophosphate (TPP) effect

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Male and female rats (156 each) were made thiamin-deficient by feeding a semi-purified diet devoid of thiamin. They were then repleted with various test diets containing chicken which had been preserved by one of four methods: frozen, thermally processed, electron or gamma irradiated. All repletion diets contained carefully controlled (marginal or high) levels of thiamin. Recovery rates were monitored by growth (weight gain) and measurements of a thiamin-dependent blood enzyme (erythrocyte transketolase). The responses of rats fed

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20. irradiated chicken were similar to those fed frozen or thermally processed chicken. No evidence was found of antithiamin substances in either gamma or electron irradiation sterilized chicken.

ABSTRACT

Male and female rats (156 each) were made thiamin-deficient by feeding a semi-purified diet devoid of thiamin. They were then repleted with various test diets containing chicken which had been preserved by one of four methods: frozen, thermally processed, electron or gamma irradiated. All repletion diets contained carefully controlled (marginal or high) levels of thiamin. Recovery rates were monitored by growth (weight gain) and measurements of a thiamin-dependent blood enzyme (erythrocyte transketolase). The responses of rats fed irradiated chicken were similar to those fed frozen or thermally processed chicken. No evidence was found of antithiamin substances in either gamma or electron irradiation sterilized chicken.

PREFACE

The experimental portions of the study covered in this report were conducted during the period of 1 September 1978 - 1 February 1979. All raw data are being stored at Letterman Army Institute of Research. Anyone wishing to examine the raw data or to obtain copies of tables containing individual values, may do so by contacting: Commander, Letterman Army Institute of Research, ATTN: SGRD-ULZ, Presidio of San Francisco, California 94129.

In addition to the personnel listed on page vi, the authors gratefully acknowledge the assistance of: LTC John T. Huber (MOBDES) who helped summarize and interpret animal weight data; Ms. Anne Regh and Ms. Marie Rogers, typists; and Ms. Lottie Applewhite, LAIR technical editor.

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REPORT OF THE QUALITY ASSURANCE UNIT

Summary and Conclusions: This study and draft of the final report dated 30 April 1979 have been examined and found to have been conducted as described in the protocol, its addendum, and in the standard operating procedures. The data have been audited and the reported results were found to accurately reflect the raw data.

Inspections: Written records were not made of inspections until 20 March 1979. On that date record was made to the best of memory of previous inspections. Following that date written record was made of all inspections at the time of inspection.

16 October 1978: Inspected animal rooms and feeding and weighing operations. No recommendations were made.

November 1978: Inspected erythrocyte transketolase assay. No recommendations were made.

February 1979: Mr. Paul Waring advised the Quality Assurance Unit of a downward shift in the recovery of the controls used in the erythrocyte transketolase assay from December 12 onward to the conclusion of the study. He provided the Quality Assurance Unit with documentation and speculated that the samples and controls may have been warmed during a defrosting of the freezer in which they had been stored. He advised management that data collected from December 12 onward to the conclusion of the study be multiplied by a correction factor of 1.16. The Quality Assurance Unit requested an amendment to the standard operating procedure.

26 July 1979 to 1 August 1979: Draft of the report dated 30 April 1979 was reviewed and the data audited. The amendment to the erythrocyte transketolase requested in February had not yet been received. This was requested and received. This draft accurately described the methods and standard operating procedures used and the reported results accurately reflected the raw data.

Present Recommendation to Management: An archive unit needs to be established, in which to store the data.

William R. Wise, Jr

WILLIAM R. WISE, JR.
QUALITY ASSURANCE MONITOR
30 Aug 1979

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Fort Detrick, MD

Signatures of Principal Scientists
Involved in the Study

We, the undersigned, believe the study described in this report to be scientifically sound and the results and interpretations to be valid. The study was conducted to comply, to the best of our ability, with the Good Laboratory Practice Regulations for Nonclinical Laboratory Studies outlined by the Food and Drug Administration.

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INTRODUCTION

The testing of control and irradiated meats for antimetabolite activity against vitamins B_1 and B_6 is a requirement of the protocol entitled "Animal Feeding Protocol for Irradiation Sterilized Test Foods" originated by the Office for the Wholesomeness of Irradiated Foods, US Army Medical Research and Development Command (USAMRDC), dated 21 October 1975.

The purpose of the study reported here was to determine whether irradiation (gamma or electron) or thermal processing of chicken produces factors which are antagonistic to vitamin B_1 (thiamin) in the diet of rats.

The protocol for the antithiamin study specified that rats were to be made deficient in thiamin (according to a pre-set weight gain criterion). They were then to be repleted with various chicken-containing or semi-purified diets which had identical (high or low) thiamin contents and the recovery rates monitored. A decreased recovery rate in animals fed irradiated meat relative to those fed control meat would indicate the presence of an antithiamin substance.

Resumption of weight gain was the obvious indicator of recovery from thiamin deficiency. The other (more sensitive and specific) parameter of thiamin status was specified to be erythrocyte transketolase (ETK) activity. This enzyme is active only in the presence of a thiamin derivative, thiamin pyrophosphate. The activity of the enzyme drops rapidly in red cells of animals fed diets deficient in thiamin. Also, *in vitro* addition of thiamin pyrophosphate (TPP) cofactor produces a greater (%) increase in enzymatic activity in hemolysates from deficient animals than from nondeficient controls. This *in vitro* stimulation, "TPP effect," is considered to be indicative of the percent apoenzyme which is not saturated with cofactor.

The protocol specified that the meat diets contain 35% test meat (on a dry weight basis). Furthermore, it specified that each meat be tested at 2 levels of vitamin intake, a marginal level and a high level. The high thiamin diets were included to determine whether or not any antithiamin substances (if detected) could be overcome by additional vitamin.

All test meats originated from one lot of chicken which had been heated to inactivate enzymes. This lot was divided into four parts and each was further prepared for storage by one of the following treatments: 1) frozen (control), 2) canned (thermally processed), 3) gamma irradiated, 4) electron irradiated. The last three treatments produce shelf-stable products and are known to cause decreased vitamin content. Finally, the protocol specified the inclusion of groups fed dry, semi-purified diets without chicken.

The original protocol had specified that the two thiamin levels be 5.0 and 20.0 mg thiamin/kg diet. However preliminary studies in this laboratory suggested that there was little difference in ETK response in thiamin-deficient rats repleted at 5.0 or 20.0 mg/kg. (For further comments concerning the levels of thiamin in repletion diets, see the previous report from this laboratory on the antithiamin properties of irradiated beef (1)). In the present study, the marginal thiamin level was lowered still further to 3.0 mg/kg diet to enhance the differences in response between the high and low vitamin groups and thus to ensure that any antithiamin properties (if present) could be detected.

METHODS

Processing of Chicken. All test meats were supplied by the U.S. Army Natick Research and Development Command, Natick, MA. They were processed according to the procedure outlined in Appendix A of the protocol described in paragraph 1 of page 1.

Enzyme Inactivation: Chicken breasts, thighs and legs were deboned, skinned and ground, then mixed with sodium chloride (7.5 g/kg chicken), sodium tripolyphosphate (3.0 g/kg chicken) and crushed ice (30 g/kg chicken). The ground chicken was then placed in fibrous casings and pressed into wire cages. It was cooked in this form for two hours at a Dry Bulb Temperature of 63° to 68°C and a Wet Bulb Temperature of 46° to 52°C, after which it was cooked at temperatures up to 88°C until the internal temperature reached 73° to 77°C. After cooking, the chicken was spray washed for 20 to 30 minutes with cold tap water and then refrigerated.

All chicken, except that to be electron irradiated, was placed in cans sealed under vacuum and frozen. Chicken to be electron irradiated was placed into rectangular pouches, sealed under vacuum and frozen until irradiated. Cans of chicken to be used as the frozen control were not processed further but remained frozen until used. To prepare thermally processed chicken, cans of chicken were heated to 115.6°C for 159 minutes according to conventional commercial practice resulting in a sterilization value of not less than Fo=6.0.

Gamma Irradiation: Chicken packaged in cans was irradiated in the frozen state using the Co-60 facility of the Radiation Laboratory at the US Army Natick Laboratories, Natick, Massachusetts. The dose of radiation absorbed was between 4.7 and 7.1 million rads (Mrads).

Electron Irradiation: Chicken packed in pouches was irradiated (while frozen) by the electron linear accelerator (Linac) facility of the Radiation Laboratory at the US Army Natick Laboratories, Natick, Massachusetts. The electrons were delivered in pulses of approximately 5 microseconds and with a pulse repetition rate of approximately 180 pulses per second. The electron energy spectrum was allowed to

peak between 9 and 10 million electron volts (MeV) during irradiation of the chicken with a full width of half maximum of 0.5 MeV or less.

Animal Care

Male and female weanling rats (156 each) were purchased from Charles River Breeding Laboratories, Wilmington, Mass. Each animal was identified by ear tag and individually caged in a room with a 12-hour light/dark cycle. All were given ad libitum water and fed a semi-purified diet (Table 1) containing 20.0 mg thiamin/kg diet.

The schedule and diet codes for the studies are outlined in Table 2. After one week of quarantine and adaptation (Phase 1), 24 rats were selected at random to remain on diet A. All other animals were placed on diet B, which was identical to diet A except that thiamin had been omitted (Phase 2). Growth was monitored throughout the study by thrice weekly weighings. Animals on diet B were considered to be deficient when the average daily weight gain was less than 0.5 grams. The deficient animals were then randomly divided into 11 groups of 12 animals each. The 24 diet A animals were also divided into 2 groups of 12 each. One group of 12 diet A rats and one group of 12 diet B rats were bled by cardiac puncture and removed from the study. The remaining 10 groups of deficient animals were placed onto 10 different repletion diets (C-L) and the remaining diet A group was continued on diet A. The repletion period (Phase 3) lasted 4 weeks.

Diet Preparations

Proximate analyses of the chicken (crude fat, protein, moisture, and ash) were done by standard methods (2). Calcium and phosphorus determinations were also done (3,4). Thiamin assays were done by a microbiological method which utilizes Lactobacillus viridescens as the test organism (5). The results of these assays are summarized in Table 3.

As specified by the protocol, the meat diets were formulated to contain 35% (dry weight) chicken. The fat and protein levels of the semi-purified diets (Table 1) were adjusted to be similar to the meat diets, based on calculations from proximate analysis data. For each of the meat treatment groups (E through L) a dry premix with fat and protein omitted and containing the proper amount of thiamin was prepared in advance. When mixed with the corresponding meat (35% dry weight basis) the complete diets contained the specified levels of thiamin and were similar to the semi-purified diets, except that ground chicken replaced the casein, lard, and corn oil. Analyzed thiamin contents of repletion diets are given in Table 4. The calcium/phosphate ratio of the meat diets was calculated to be 1.294 compared to 1.25 in the semi-purified diets; therefore, no adjustment was made in these two minerals.

Each meat was ground through a 1/4 inch plate and mixed with its

corresponding dry premix in the specified ratio. The diets were prepared no longer in advance of feeding than 24 hours. Each feed jar was weighed when placed into and removed from the cage to allow estimates of food consumption. The diets remained in the cages no longer than 48 hours before being replaced by fresh diet in clean jars. Feed jars containing each diet were also placed in empty cages to serve as evaporation controls.

Blood Sampling and Analyses

Blood samples were obtained by cardiac puncture from all rats on days 7, 14, and 27 of repletion. (The last sampling was set at day 27 rather than day 28 to avoid Thanksgiving holiday.) The animals were anesthetized with penthrane gas and samples (1.5 ml each) were collected into EDTA-containing syringes. Hematocrit determinations were done in duplicate on each sample. Aliquots of each were centrifuged, the red cells were washed, hemolyzed and stored frozen until assayed. Erythrocyte transketolase (ETK) activity was determined in the presence and absence of added thiamin pyrophosphate (TPP) by a method based on that of Smeets et al. (6) but adapted for the autoanalyzer (Waring, P.P., et al., In Preparation). Enzymatic activity was expressed as ETK = I.U./ml packed red cells where I.U. = μmole glyceraldehyde-3-phosphate (G3P) produced per minute at 37°C. The stimulation in activity in the presence of TPP was calculated as:

$$\text{TPP Effect} = \frac{[\text{ETK stimulated} - \text{ETK unstimulated}]\ (100)}{\text{ETK unstimulated}}$$

Quality control summaries are presented graphically in Figures 1 and 2. In both figures, the dotted lines represent 2 standard deviations on either side of the means (solid lines). Figure 1 illustrates the day-to-day chart unit response to 3 different concentrations of G3P added to a carrier hemolysate. (The carrier was a composite of blood samples prepared in advance and frozen in aliquots for use throughout the study). In addition to the carrier, two other hemolysates were prepared in advance and frozen in aliquots for quality control purposes: FC, a composite of blood samples from non-deficient rats and FD, a composite of blood samples from thiamin-deficient rats. Figure 2 illustrates the day-to-day ETK activity values (and the coefficients of variation) obtained for FC, FD and the carrier. All three controls dropped by an average of 16% on assay days 17-27 compared to the first 16. (The reason for the drop is not known, but a transient warming during freezer defrosting is suspected). Since the chart unit responses remained constant throughout the study and apparent ETK activity in all three control hemolysates dropped equivalently, their values in Figure 2 and all samples assayed on those days have been adjusted up by 16%.

Data Acquisition and Handling

Animals were weighed using an electronic balance interfaced with a programmable calculator. The weight data were permanently printed

on paper tape and recorded on a miniature tape cartridge. The information on the magnetic tape was then transferred to a minicomputer (Data General Eclipse C330) to be processed and released in report format. Programs for transferring and processing the data were developed locally in the Information Sciences Group.

Erythrocyte transketolase data were recorded in digital print-out form, on paper tape from the auto-analyzer. These data, as well as the hematocrit values, were manually transferred to the computer through a remote terminal. All programs to process enzymatic data were developed by personnel in the Division of Nutrition Technology.

Statistical Analysis of Erythrocyte Transketolase Data

The measurements taken on the three sample days (7, 14, and 27) were analyzed separately for the effects of the design variables (i.e. diet, level of thiamin and sex) along with their interactions on ETK, ETK-stimulated and TPP effect.

Based on the assumptions of normality, statistical independence and the equality of subgroup variances, three-way analyses of variances were performed with a packaged computer program, BMDP Biomedical Computer Program P2V (7). The analyses revealed that the effects of diet and level of thiamin were different for the different sex groups. Thus it was decided to separate the analyses further between the sex groups and a two-way analysis of variance was performed on each of the three dependent variables to determine the significance of the two main effects (diet and thiamin level) and their interactions.

The following model was used in the two-way analyses of variances:

$$y = m + a_i + b_j + ab_{ij} + e$$

where y = the observed ETK
m = overall mean effect
a_i = effect due to diet group
b_j = effect due to thiamin level
ab_{ij} = effect due to diet by thiamin level interaction
e = error term

Dunnetts method of multiple comparisons (8) was used to test potentially significant differences between specific treatment groups.

RESULTS

Observations on Growth

Growth (weight) curves for the males are shown in Figures 3-8 and for the females in Figures 9-14. (Phases 1 and 2 are represented in Figures 3 and 9.) Groups A and B were indistinguishable for at least 10 days after the beginning of phase 2 and then cessation of growth in

animals on the deficient diet was remarkably abrupt. Both males and females met the criterion for deficiency (weight gain less than 0.5 g/day) 14-16 days after being placed on the thiamin deficient diet.

Figures 4-8 and Figures 10-14 show the growth curves for all groups on the various repletion diets. The growth curve of Group A has been included in each figure (dashed line). A striking observation was the fact that when the thiamin-deficient animals were placed on the repletion diets, all chicken-fed groups gained faster than those repleted on semi-purified diets. In fact, within two weeks all groups on chicken diets had caught up to the non-deficient group and the females had strikingly surpassed their Group A.

A few accidental deaths occurred as a result of the anesthesia or cardiac puncture. These losses, as well as the stresses of bleeding all animals, resulted in potentially misleading shifts in some growth curves after days 7 and 14. To eliminate the potentially misleading effects of animal deaths on group means, the growth data were recalculated and expressed as mean weight gain per group per day. Tables 5 and 6 summarize the average daily weight gain for the males and females for weekly periods as well as the overall means for the four weeks of repletion. No consistent effect of vitamin level on weight gain was found, nor were there any differences observed between chicken-fed groups. As pointed out above, the average gains for animals fed semi-purified diets were less than those for the meat-fed groups. The daily mean weight gains from which the numbers in Tables 5 and 6 were calculated are included in Tables 7 and 8.

Results of Erythrocyte Transketolase (ETK) Analyses

Erythrocyte transketolase (ETK) data (unstimulated) are summarized in Table 9 and presented graphically by day in Figures 15-20. In each figure, the far left bar represents Group A (non-deficient animals). Group B means (representing the deficient animals at day 0, before repletion) have been dotted in to aid visual comparisons. The bars are paired together according to food group and in each pair the open and diagonally hatched bars represent low and high vitamin level, respectively.

Before repletion, the deficient animals (Group B) had ETK activities 20 - 25% of the levels observed in non-deficient controls. From Figures 13 and 16, it is obvious that within 7 days, ETK activity of these animals had increased strikingly regardless of the repletion diet. Smaller increases were observed in all groups on day 14 and in the females on day 27. The high vitamin group in each pair of repletion diets consistently had the higher ETK activity. At 27 days, all experimental group means (both high and low vitamin levels) were still lower than Group A means (Figures 15 and 18).

In contrast to the enzymatic activities, the *in vitro* effect of TPP cofactor was not greatly influenced by thiamin status or dietary treatment (Table 10). Although the Group B males did have a higher mean

TPP effect than Group A (and also a high standard deviation), there was no difference between the corresponding female groups.

Statistical Analysis of Erythrocyte Transketolase Data

Two-way analyses of variance (diet and vitamin level as grouping factors) were done on the ETK data from Groups C - L and P values are presented in Table 11. The enzymatic parameters, ETK, ETK-stimulated and TPP effect will be discussed separately.

1. ETK and ETK-stimulated (both related to the amount of enzyme present).

Significant differences were found between the high and low vitamin groups on all three days in both male and female groups. No effect of diet was found in the males, except on day 7 ($P = 0.03$ and 0.05 for ETK and ETK-stimulated). This was probably due to the high means of Groups J and L (both high vitamin, irradiated groups) on that day. The females showed no difference between diet groups except on day 14 ($P = 0.02$ and 0.05). This effect may be attributed to the difference between Groups C and G (low vitamin: semi-purified and thermal diets). Thus there was no consistent effect of diet on ETK activity.

Since the low vitamin, irradiated meat Groups I and K were considered the crucial test groups, these two were examined more closely with respect to the corresponding frozen chicken group. In no case was an irradiated group mean found to be sufficiently lower than the frozen group to warrant further scrutiny.

2. TPP Effect (related to the % unsaturation of enzyme with cofactor).

No effect of diet was found on TPP effect. There was a significant effect of vitamin level in the males only on day 7 ($P = 0.004$). Interactions appeared in the males on day 14 ($P = 0.04$) and females on day 7 ($P = 0.05$). Thus the TPP effect appeared to be unaffected by diet or thiamin status.

DISCUSSION

In the present study, absolute ETK enzyme activity rather than % stimulation by cofactor was the more sensitive index of thiamin status. ETK in deficient animals had dropped to 20-25 of that present in non-deficient animals. The responses of animals repleted with low thiamin intake were significantly less than those of animals repleted with high vitamin intake. ETK activity in the marginal thiamin groups, thus provided valid test systems for the presence of antithiamin substances. No evidence of any thiamin antagonism was detected.

ETK activity in thiamin deficient rats increased 2-3 fold within 7 days during repletion, but still averaged approximately 60% of the

activity of non-deficient animals. These facts indicate that not all of the decreased ETK activity in deficient animals was due to an absolute loss in enzyme. Nearly half of the enzyme remained circulating as inactive, but intact, apoprotein, ready to be reactivated by the appearance (in vivo) of the vitamin. The slower rise in ETK activity after the first week of repletion is presumably limited by red cell turnover rate, i.e. maximal ETK activity would not be attained until new erythrocytes replaced all those circulating red cells which contained reduced ETK apoprotein.

In vitro addition of thiamin pyrophosphate cofactor reactivated the apoenzyme little, if any. The small TPP effects were at first disconcerting, but the phenomenon is not without precedence (9). Brin (10) has pointed out that TPP stimulation may not always be elicited in hemolysates of rat erythrocytes and that it varies greatly among different strains of rats (11). The small, variable reactivation by TPP in vitro appears to be due to a lability of apotransketolase upon hemolysis of the red cells (McGown, E.L. and M.G. Rusnak, unpublished observations). The reasons for this apparent lability are not known but are currently under investigation in this laboratory. The magnitude of the TPP effect is greatly influenced by sampling, handling and storage procedures.

Thermal processing is known to cause destruction of vitamins. Table 3 reveals that gamma irradiation is also detrimental toward the thiamin content of chicken. Electron irradiated chicken fared better, but did not retain as much of the vitamin as the frozen control. However, the destruction of thiamin (75% in thermal and gamma irradiated, 30% in electron irradiated, relative to frozen control) did not result in antivitamin substances.

CONCLUSIONS

1. Under the conditions of these studies, ETK enzymatic activity rather than TPP effect was the more sensitive indicator of thiamin status; ETK activity was highly dependent on the amount of dietary thiamin while TPP effect was largely unaffected.

2. Thiamin-deficient rats were repleted with semi-purified diets or diets containing chicken (frozen, thermally processed, gamma irradiated or electron irradiated). No difference was found among the groups in growth (weight gain) or ETK response. Specifically, neither gamma, nor electron irradiated chicken had a detrimental effect on thiamin status in rats, compared to rats fed non-irradiated (stored frozen) test meat.

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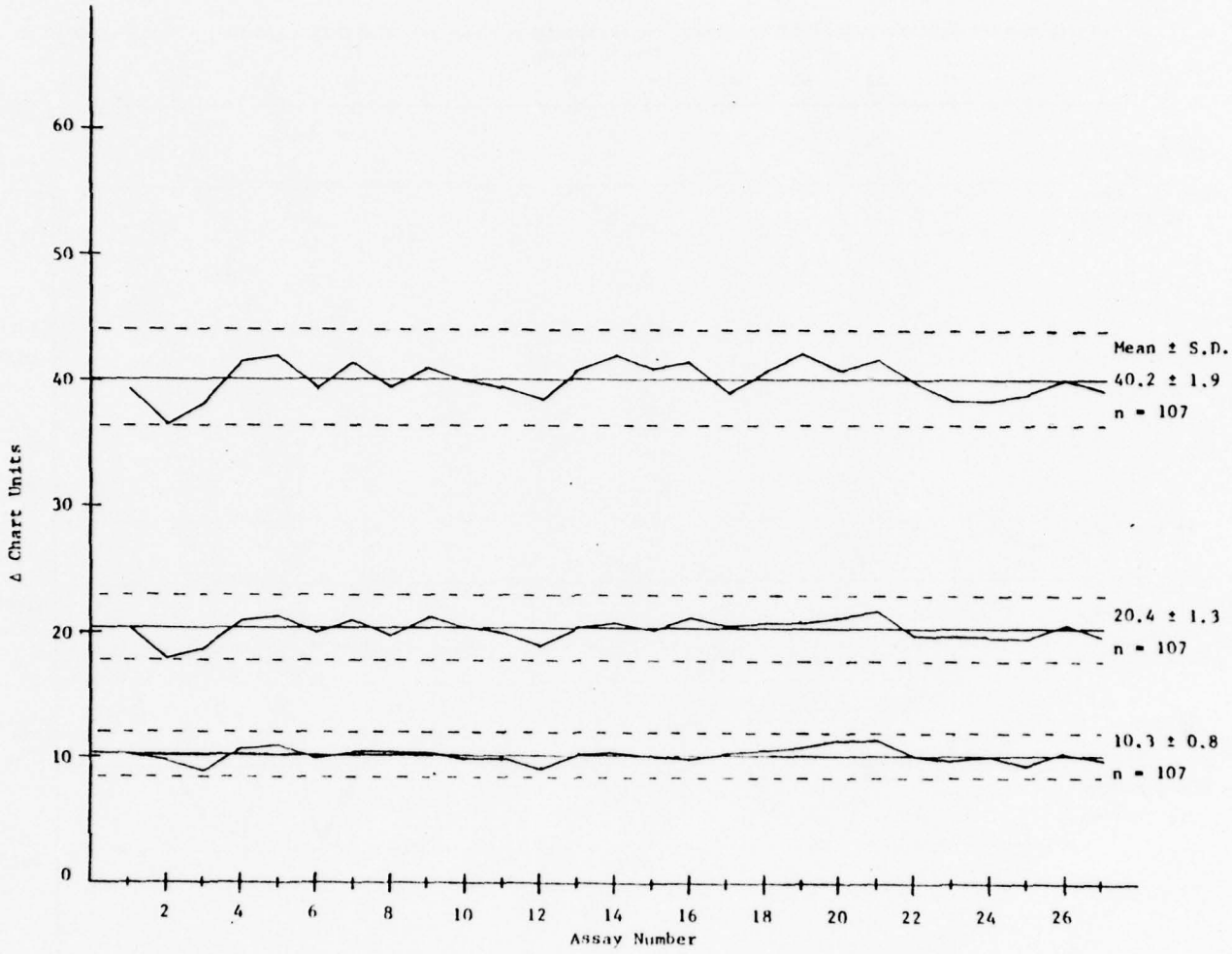

Figure 1. Day-to-Day Precision of G-3-P Internal Standards.

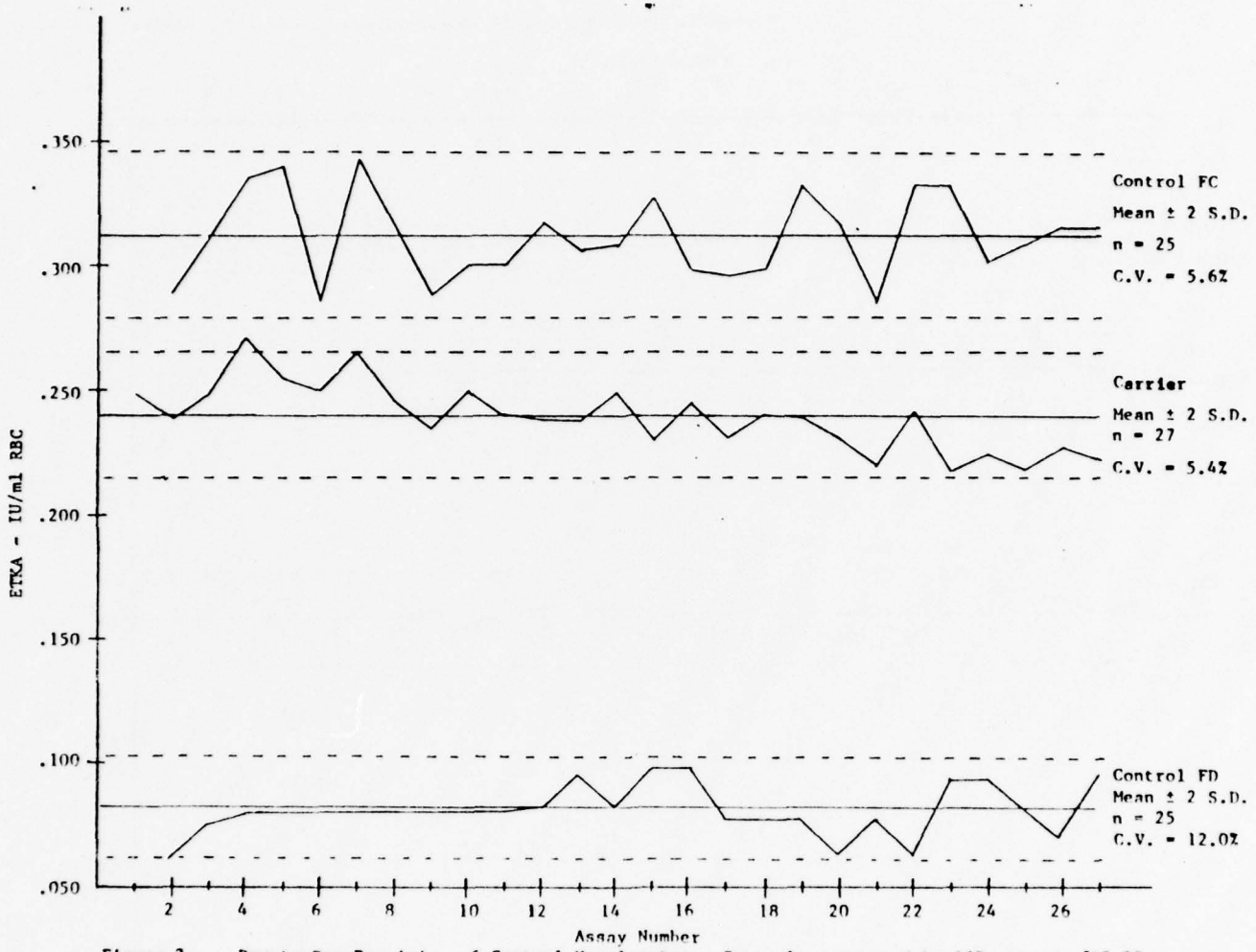

Figure 2. Day-to-Day Precision of Control Hemolysates. Controls corrected by 16%, assays #17-27.

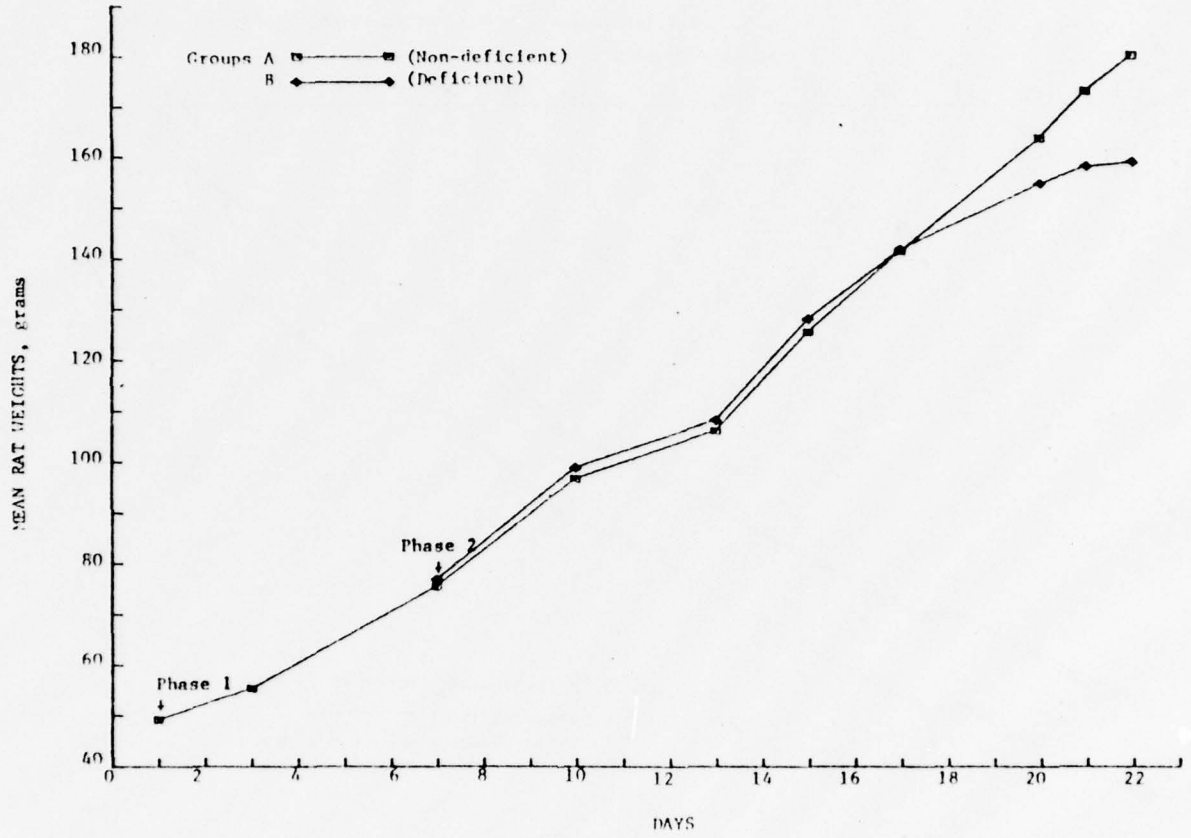

Figure 3. Growth Curve, Males, Phase 1 and 2, Groups A and B.

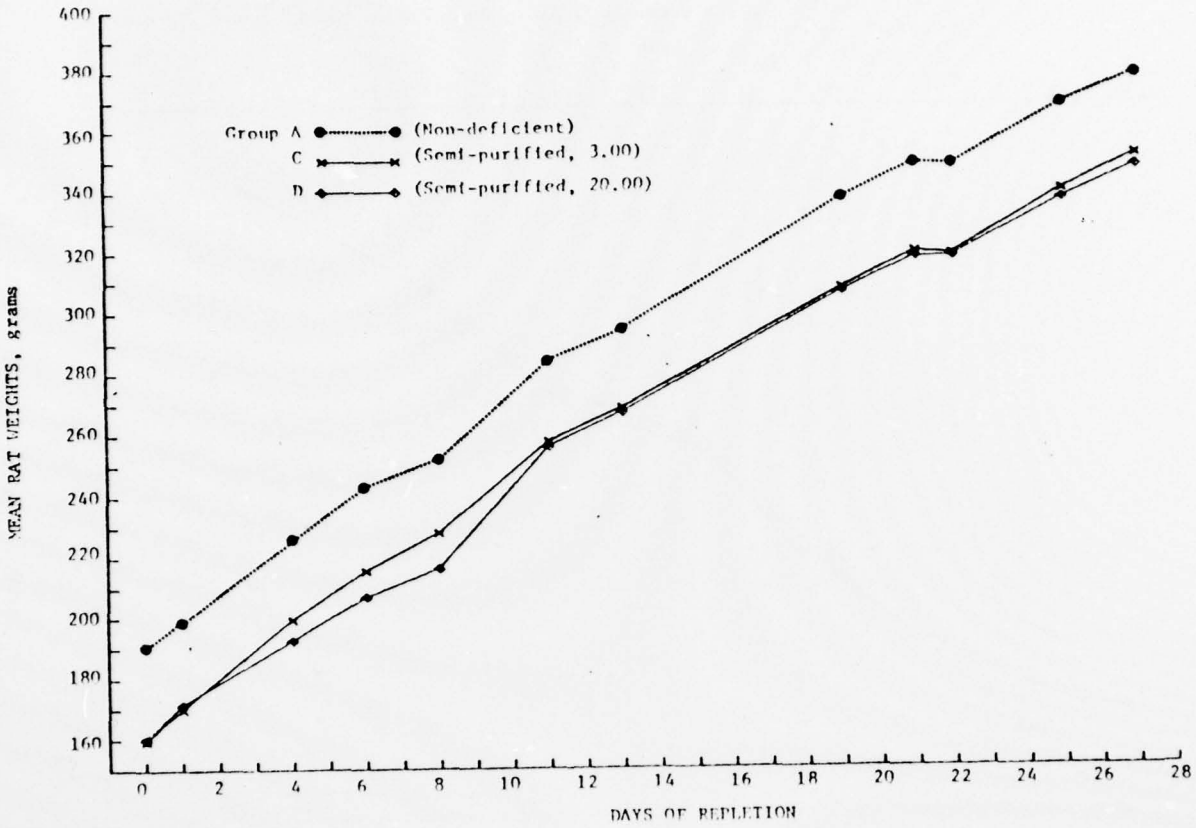

Figure 4. Growth Curve, Males, Phase 3, Groups A, C and D.

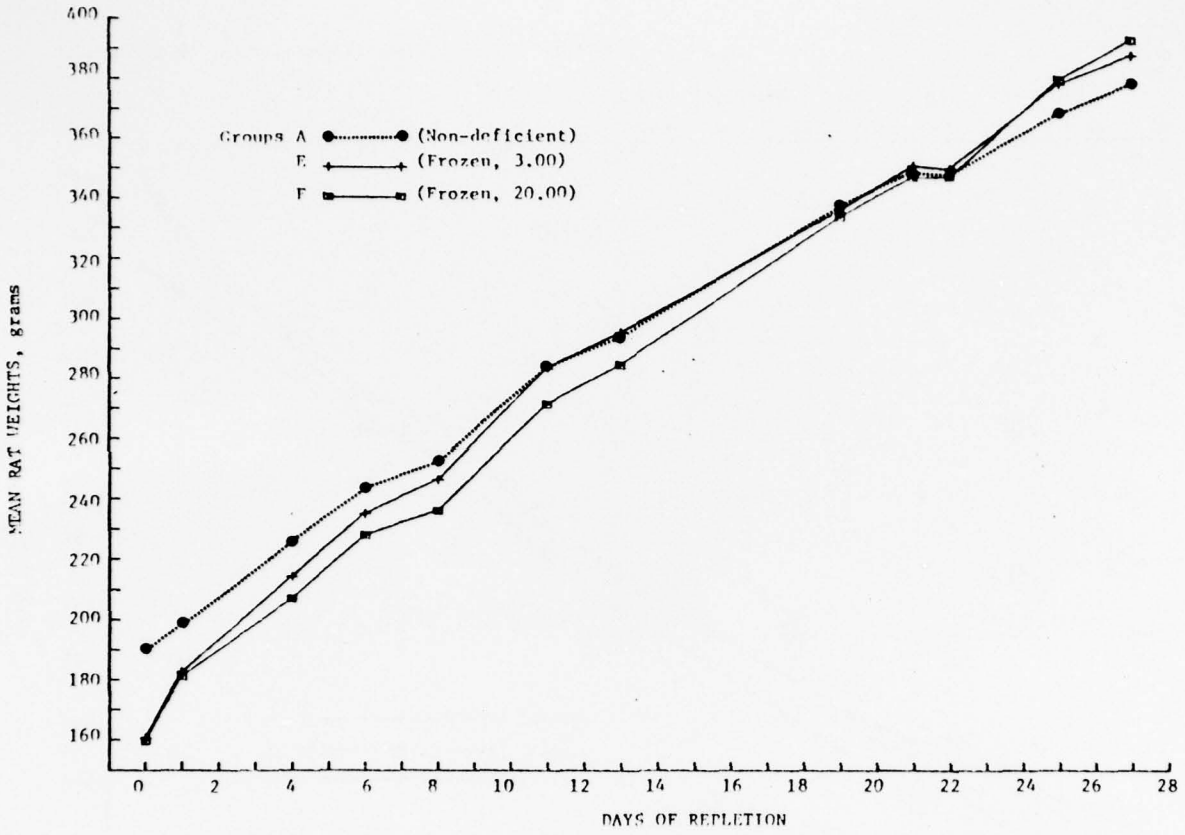

Figure 5. Growth Curve, Males, Phase 3, Groups A, E and F.

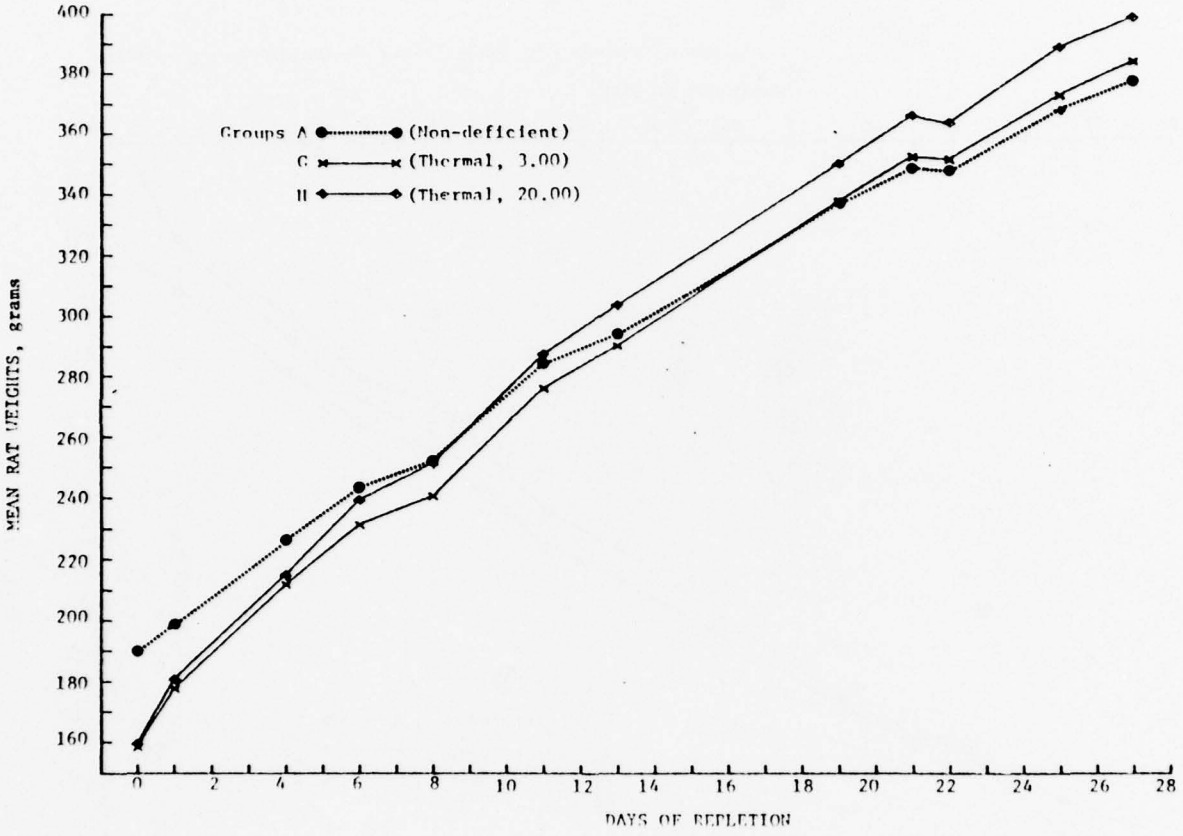

Figure 6. Growth Curve, Males, Phase 3, Groups A, C and H.

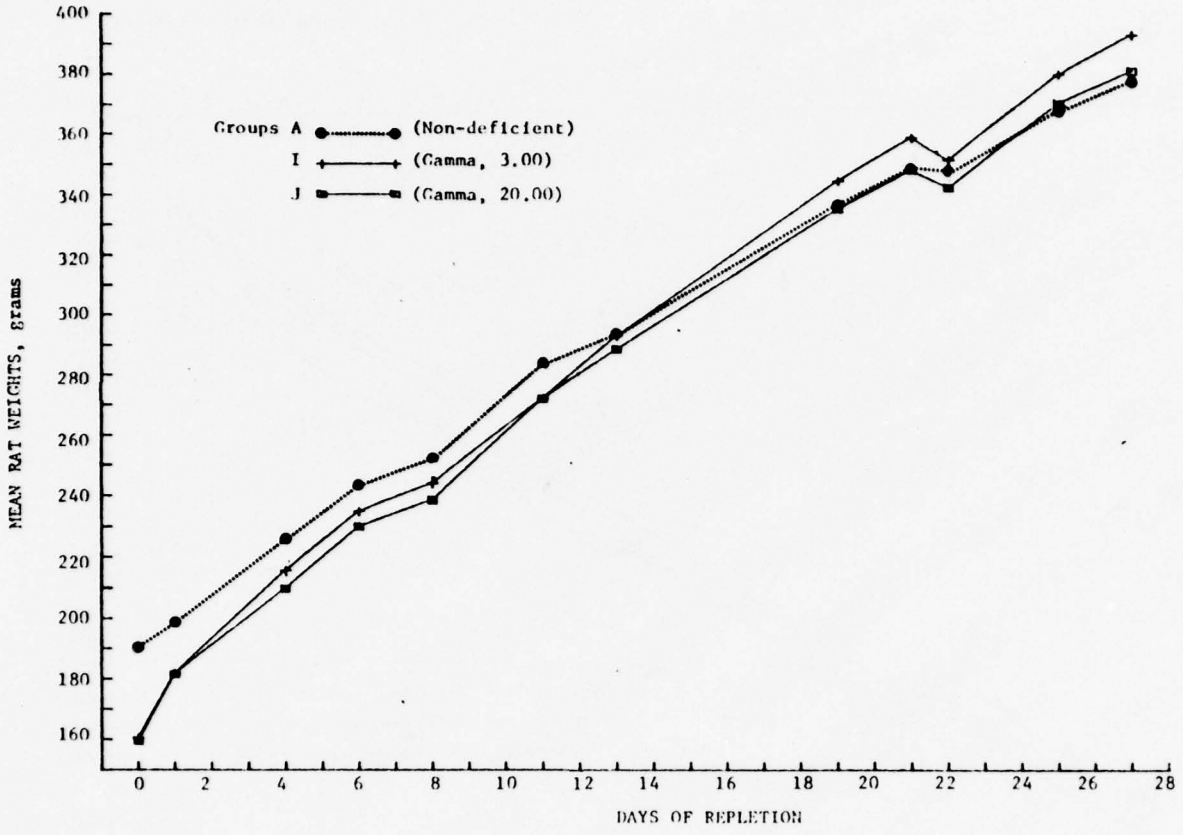

Figure 7. Growth Curve, Males, Phase 3, Groups A, I and J.

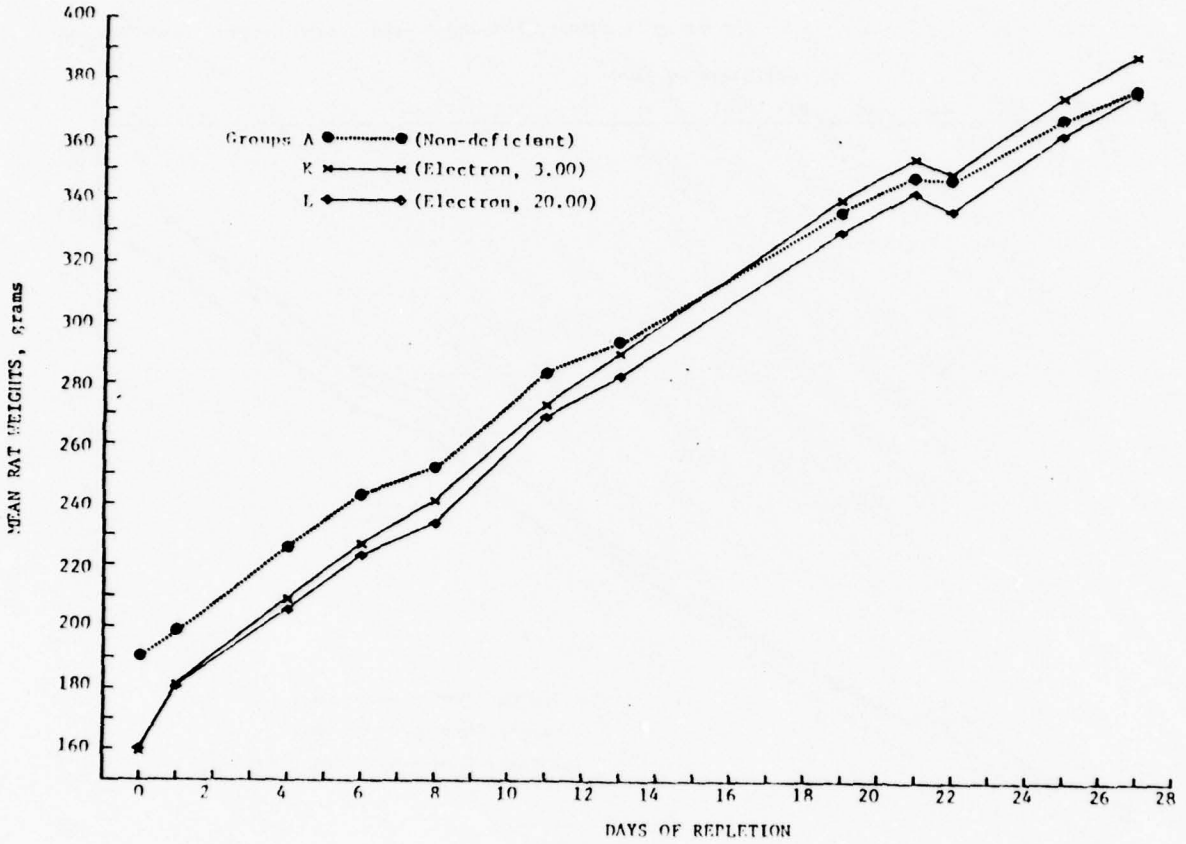

Figure 8. Growth Curve, Males, Phase 3, Groups A, K and L.

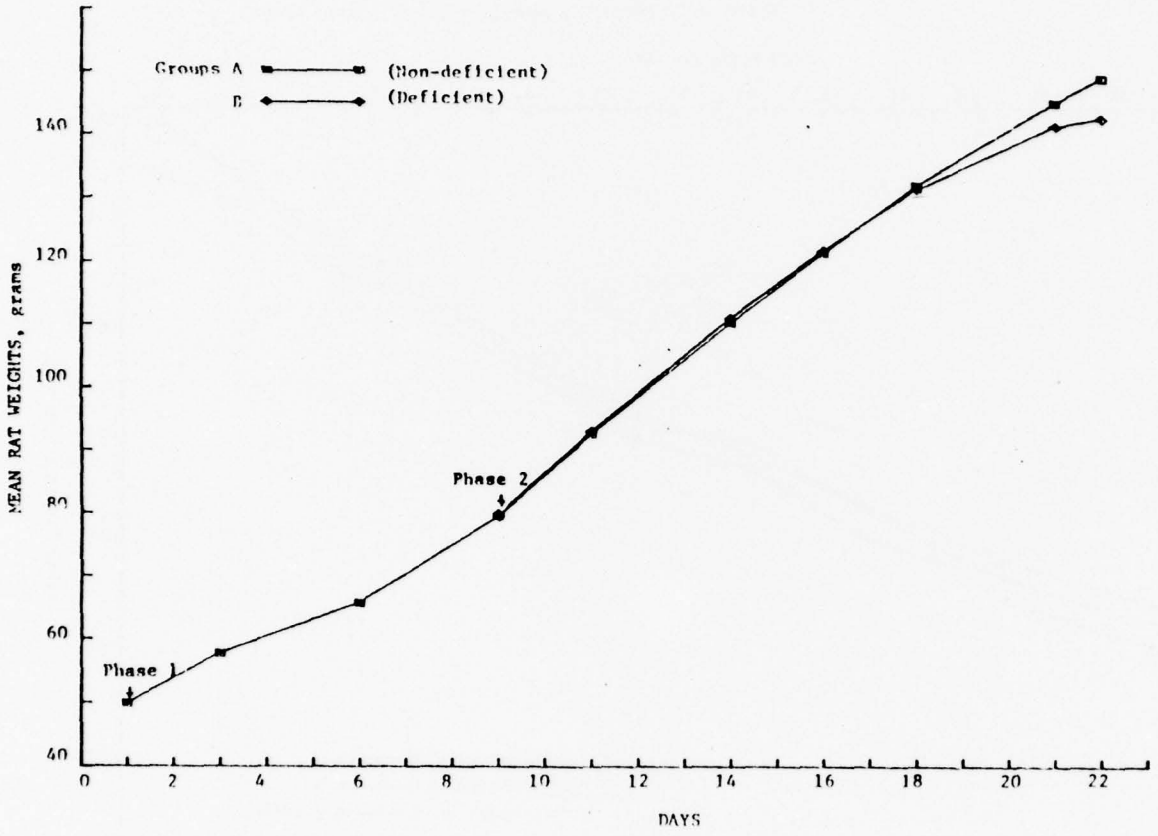

Figure 9. Growth Curve, Females, Phase 1 and 2, Groups A and B.

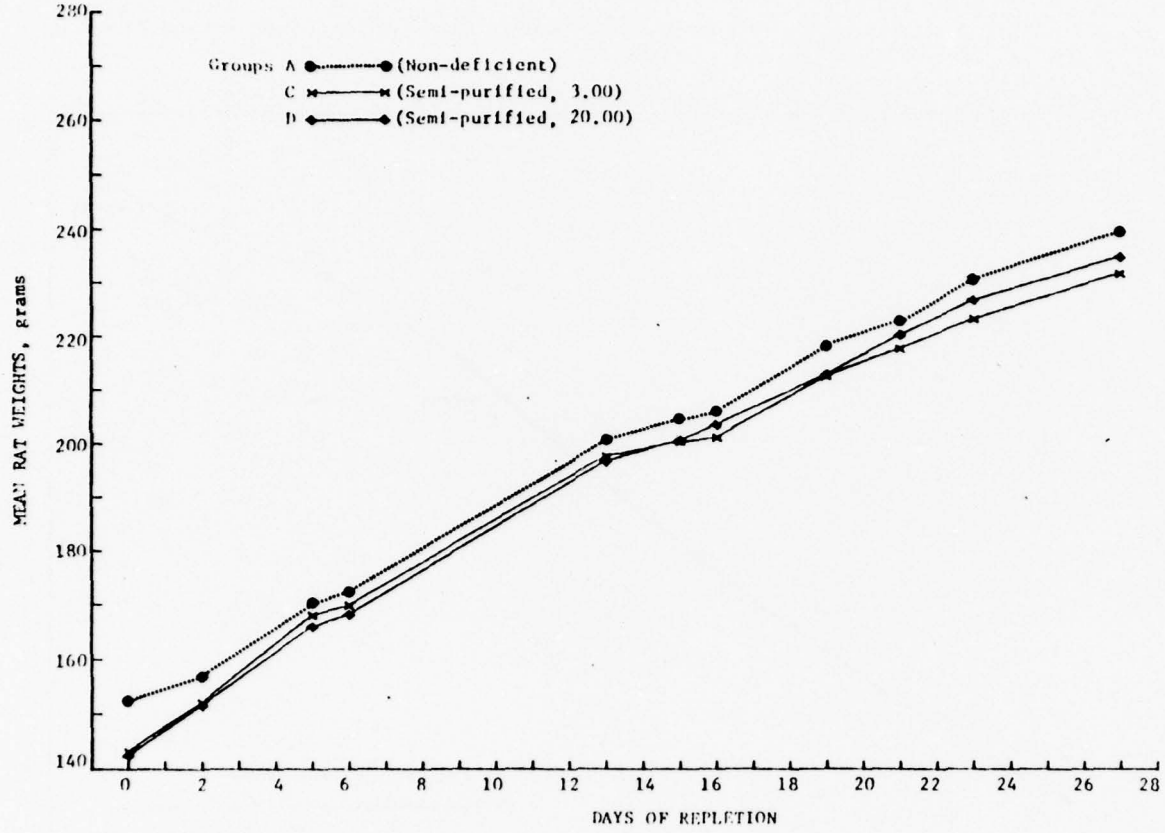

Figure 10. Growth Curve, Females, Phase 3, Groups A, C and D.

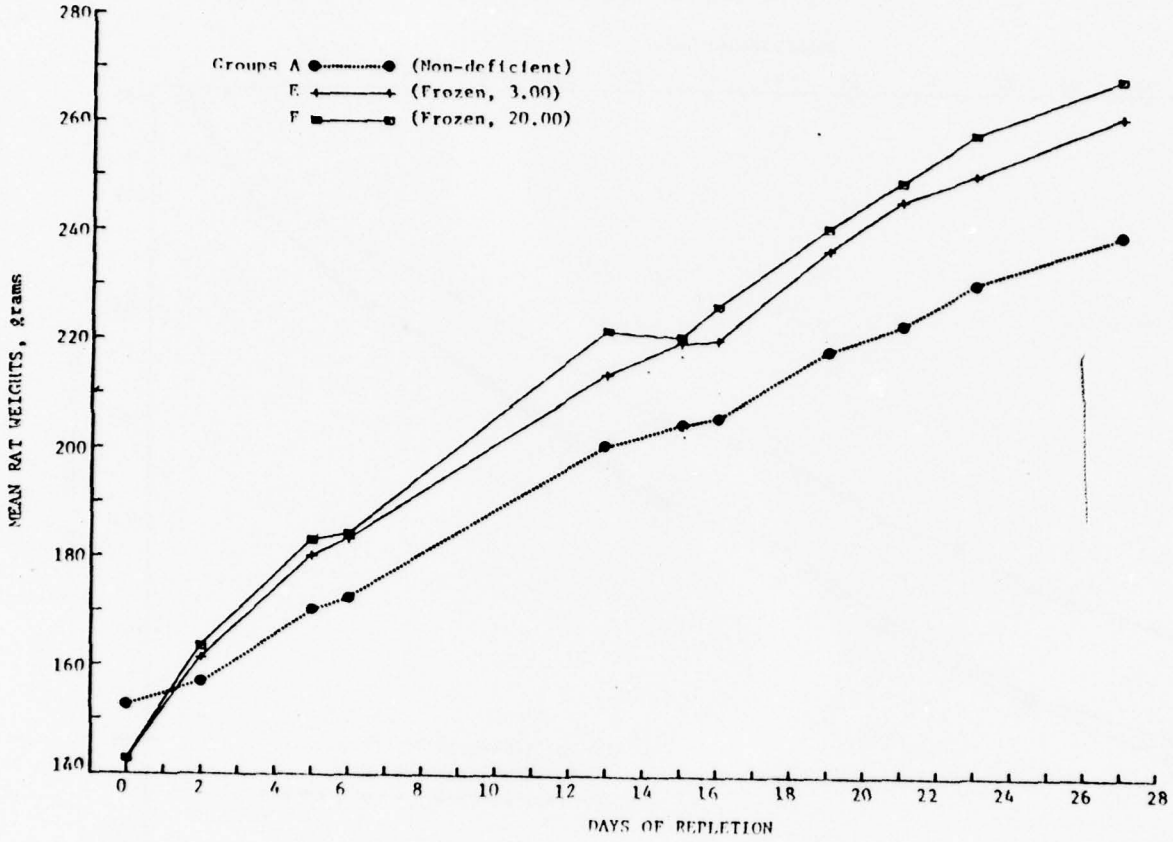

Figure 11. Growth Curve, Females, Phase 3, Groups A, E and F.

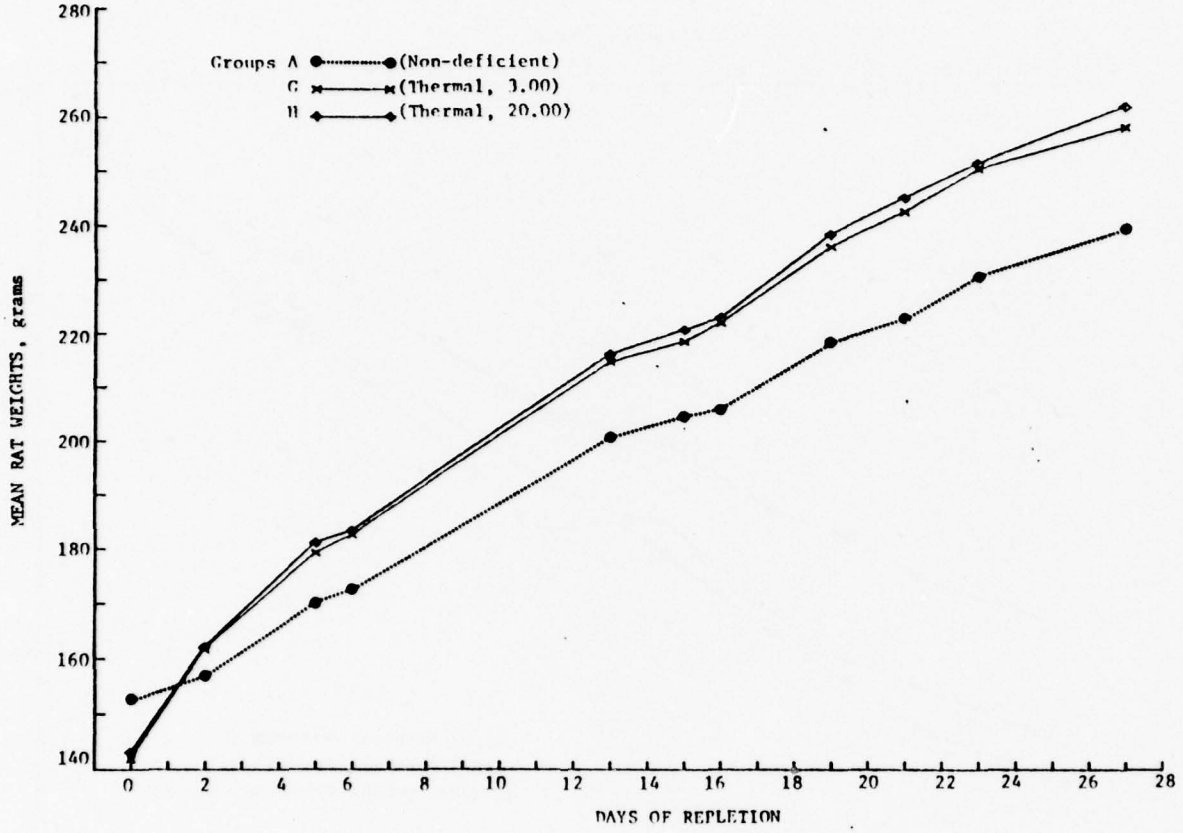

Figure 12. Growth Curve, Females, Phase 3, Groups A, C and H.

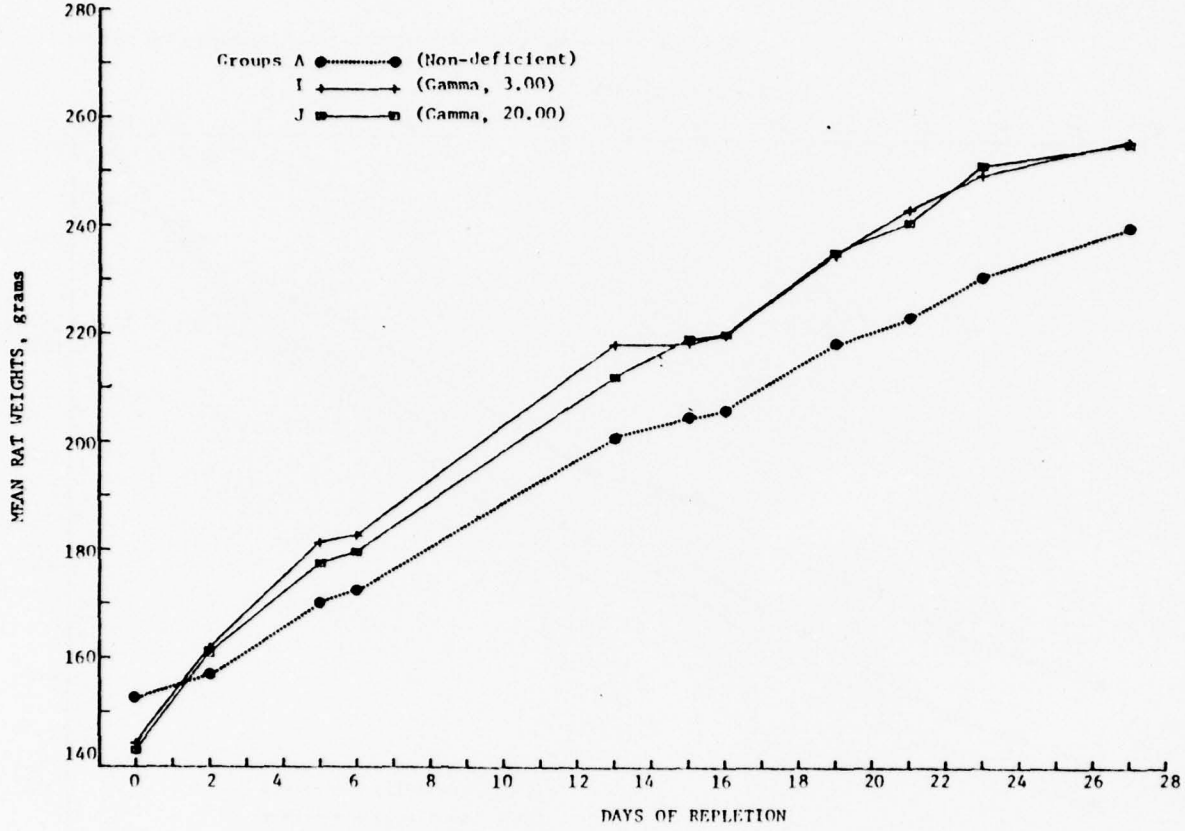

Figure 13. Growth Curve, Females, Phase 3, Groups A, I and J.

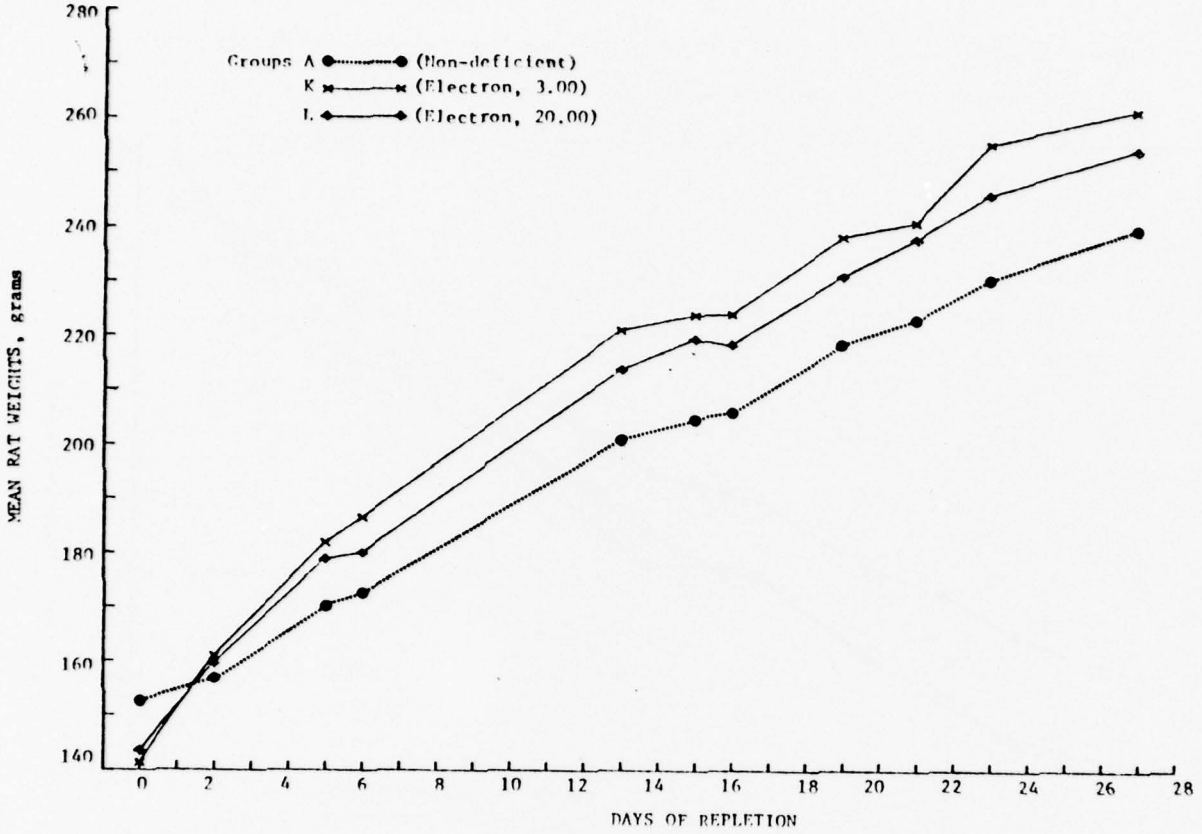

Figure 14. Growth Curve, Females, Phase 3, Groups A, K and L.

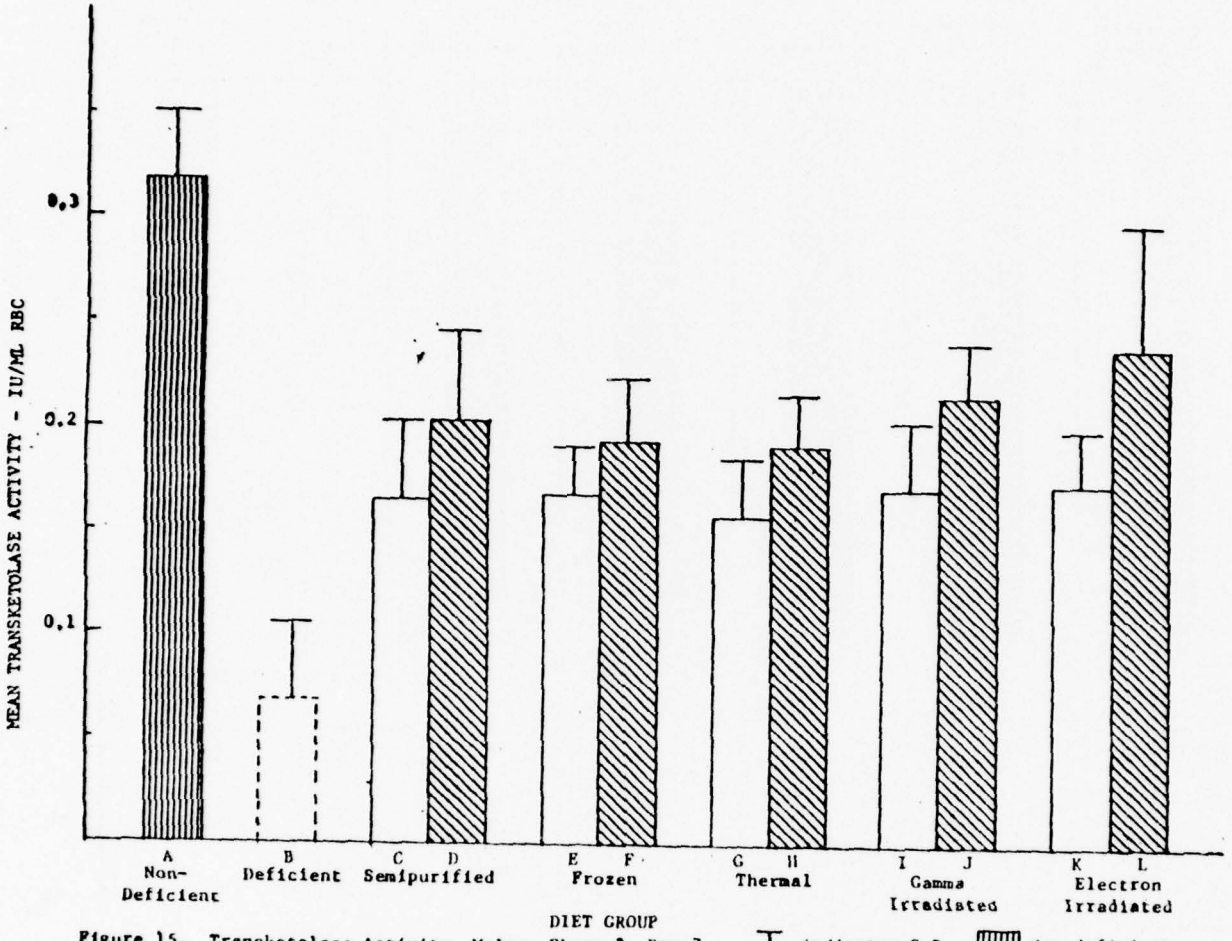

Figure 15. Transketolase Activity, Males, Phase 3, Day 7. I indicates S.D. Non-deficient group, 20 mg thiamin/kg diet. Thiamin deficient group, Day 0. 3 mg thiamin/kg diet. 20 mg thiamin/kg diet.

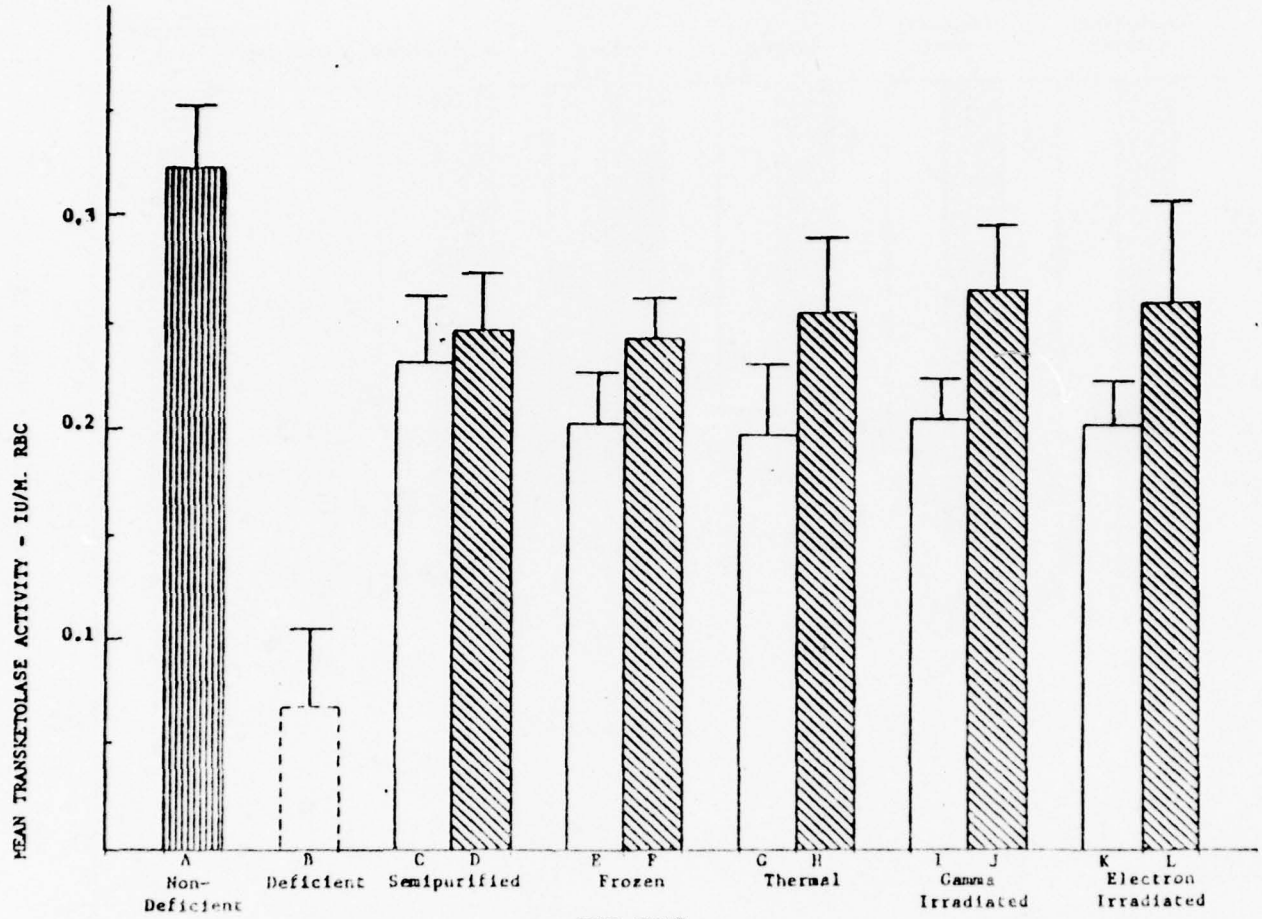

Figure 16. Transketolase Activity, Males, Phase 3, Day 14. ⊥ indicates S.D. ▨ Non-deficient group, 20 mg thiamin/kg diet. [ ] Thiamin deficient group, Day 0. ▭ 3 mg thiamin/kg diet. ▧ 20 mg thiamin/kg diet.

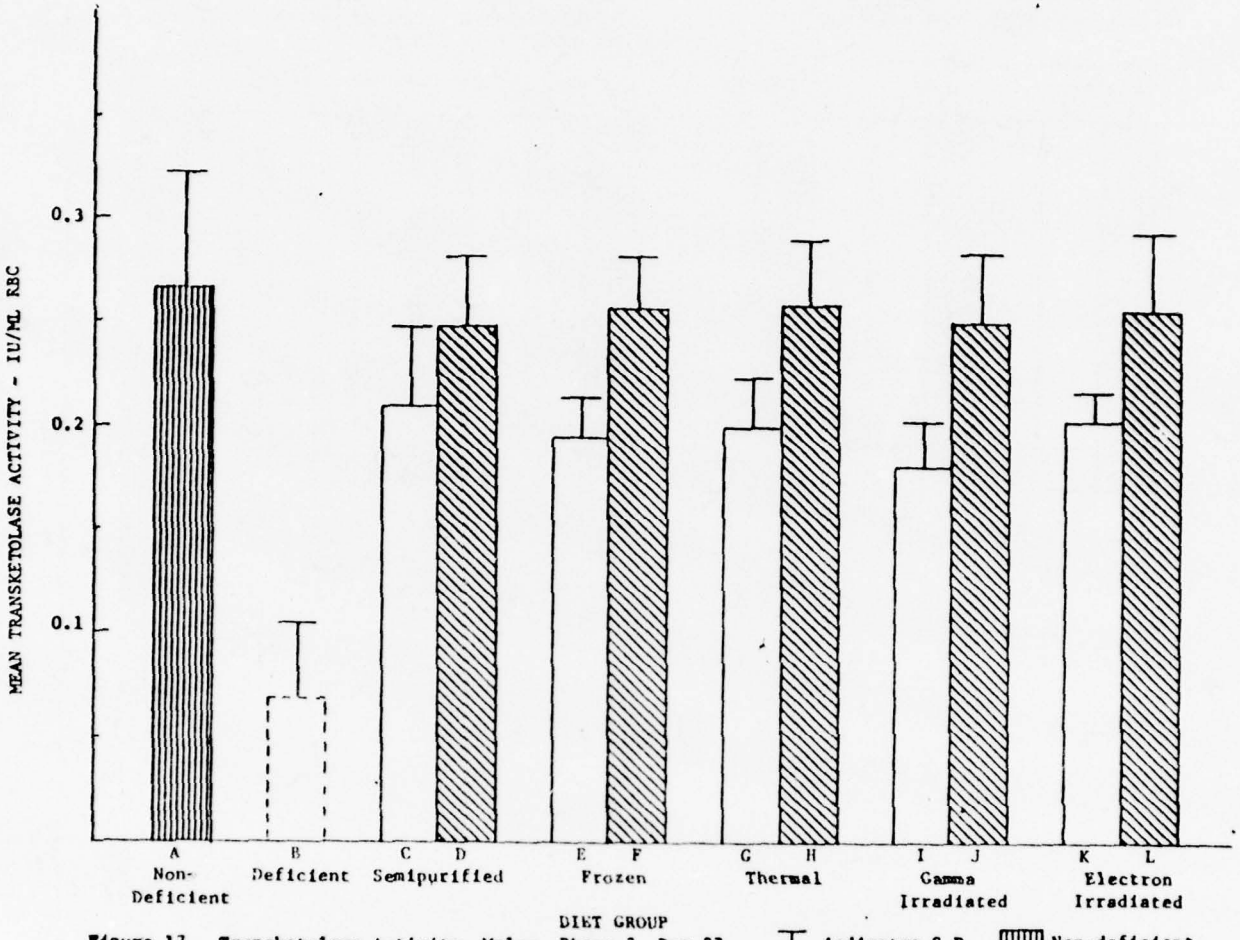

Figure 17. Transketolase Activity, Males, Phase 3, Day 27. ⊥ indicates S.D. ▨ Non-deficient group, 20 mg thiamin/kg diet. ▭ Thiamin deficient group, Day 0. ▭ 3 mg thiamin/kg diet. ▨ 20 mg thiamin/kg diet.

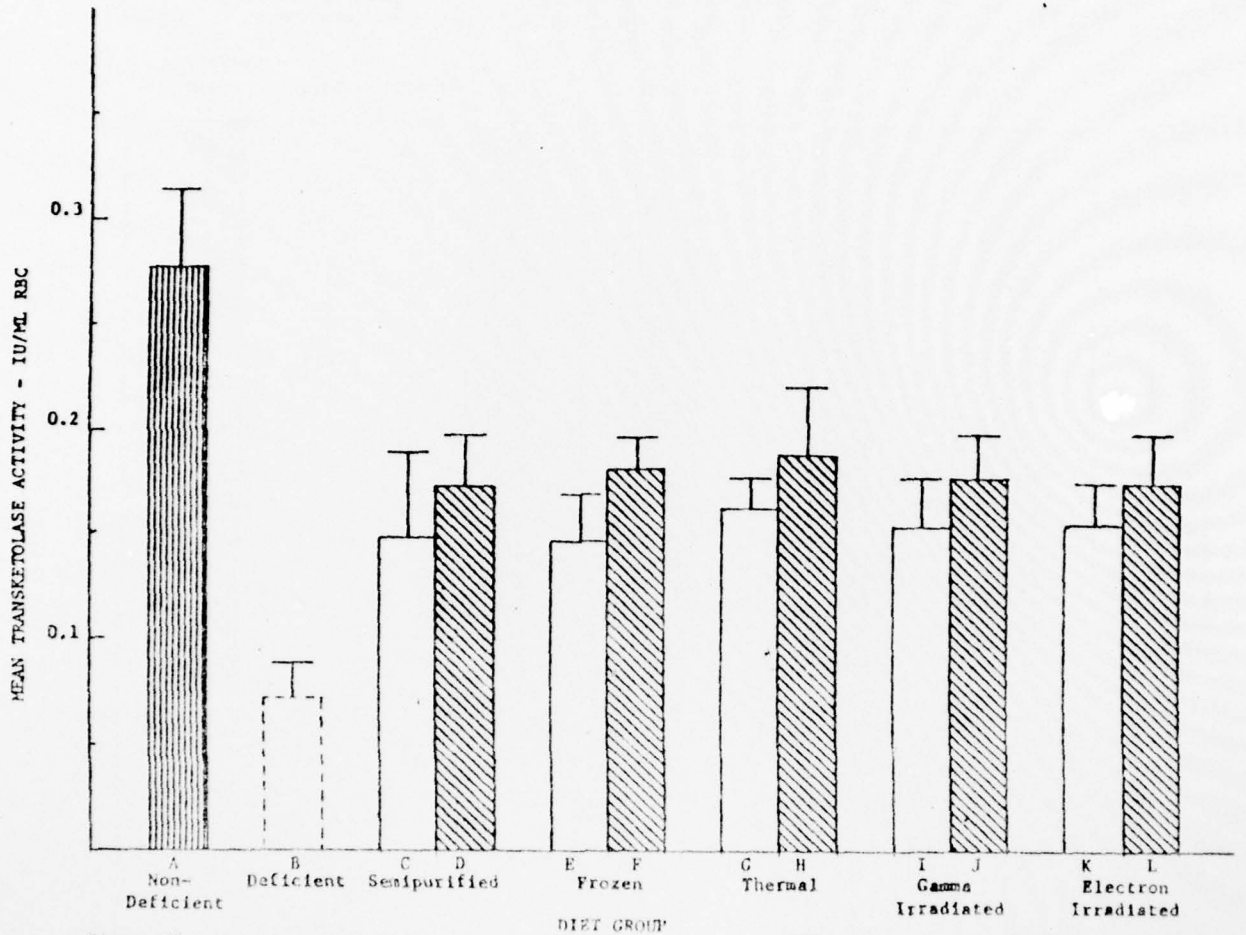

Figure 18. Transketolase Activity, Females, Phase 3, Day 7. ⊥ indicates S.D. ▥ Non-deficient group, 20 mg thiamin/kg diet. ⬚ Thiamin deficient group, Day 0, ▭ 3 mg thiamin/kg diet. ▨ 20 mg thiamin/kg diet.

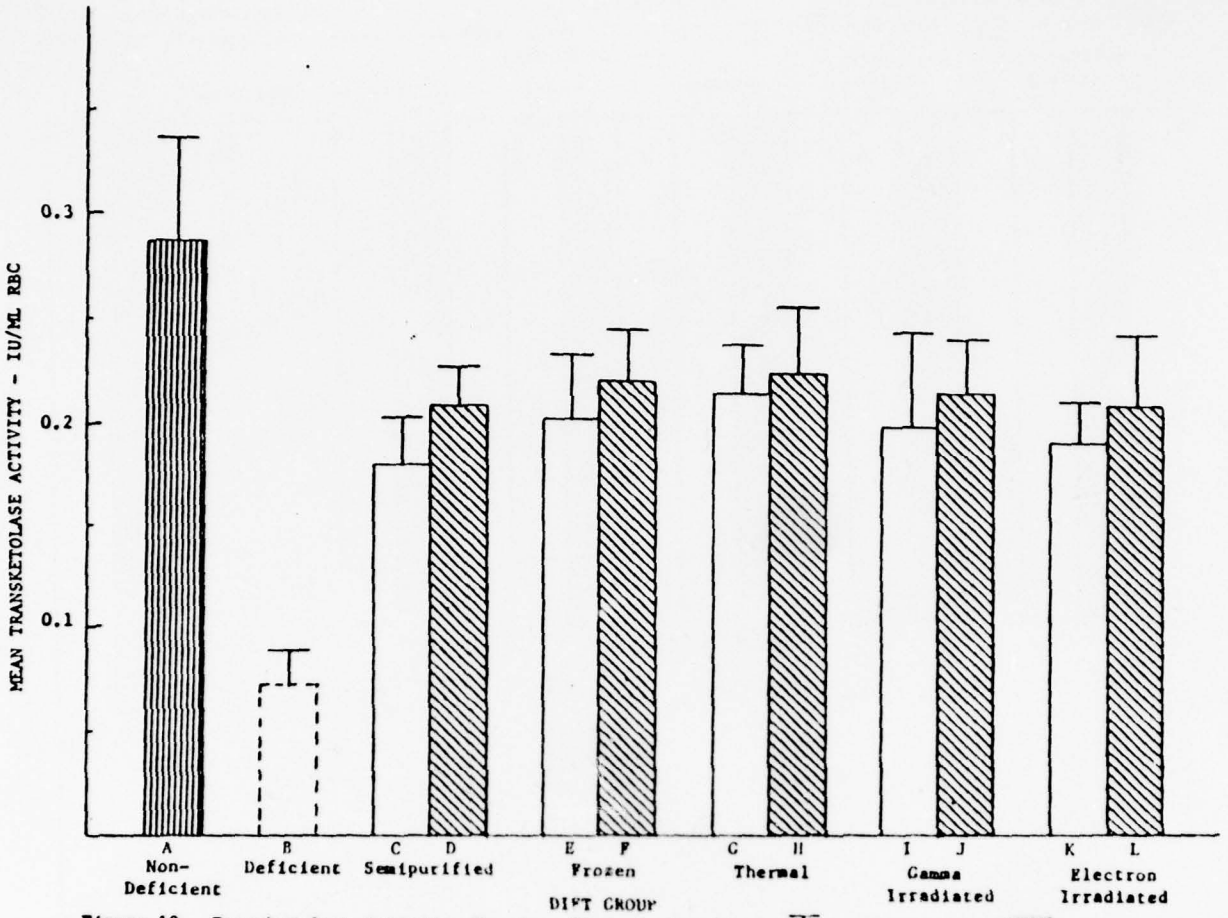

Figure 19. Transketolase Activity, Females, Phase 3, Day 14, ⊥ indicates S.D. ▨ Non-deficient group, 20 mg thiamin/kg diet. ⊏⊐ Thiamin deficient group, Day 0. □ 3 mg thiamin/kg diet. ▧ 20 mg thiamin/kg diet.

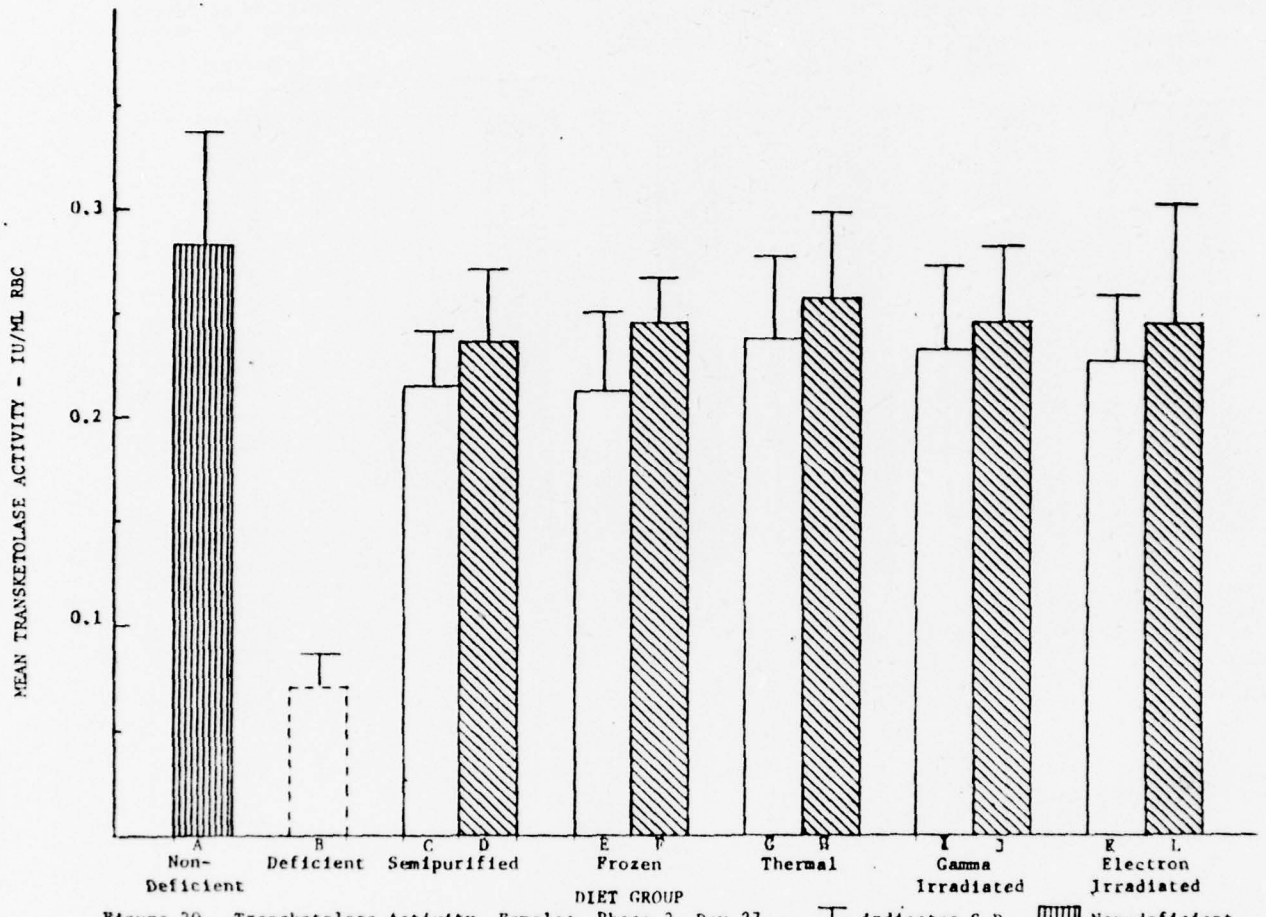

Figure 20. Transketolase Activity, Females, Phase 3, Day 27. ⊥ indicates S.D. ▥ Non-deficient group, 20 mg thiamin/kg diet. ⊏⊐ Thiamin deficient group, Day 0. ▭ 3 mg thiamin/kg diet. ▨ 20 mg thiamin/kg diet.

APPENDIX B

TABLE 1. Composition of Diets

	Semipurified %	Chicken %
Chicken (dry weight)[1]	-	35.0
Casein, vitamin free	21.8	-
Lard	8.8	-
Corn Oil	4.4	-
L-cystine	0.2	0.2
Vitamin mix[2]	2.0	2.0
Choline chloride	0.2	0.2
Mineral mix[3]	4.0	4.0
Cerolose	58.6	58.6
	100%	100%

[1]Lot labeled "Prod 3" and "Prod 3A."

[2]The vitamin premix was made up in a cellulose carrier and contributed to the final diet the following vitamins in mg/kg: gelatin coated retinal (500 IU/mg) 26; Cholecalciferol (400 IU/mg), 5; DL-α-tocopheryl-acetate powder (250 IU/g), 440; Menadione - sodium bisulfite trihydrate 1.0; Riboflavin 10; Pyridoxine • HCl 20; Niacin 60; Ca - D-Pantothenate 30; Folic Acid 2.0; Biotin 1.0; B_{12}, 0.1% triturate, 30. Thiamin • HCl was incorporated into a second premix and added to the diets to achieve the specified levels.

[3]The mineral mix contributed to the diet the following salts: in g/kg: $CaCO_3$, 4.78; $CaHPO_4$, 22.21; $NaHCO_3$, 1.164; NaCl, 1.494; K_2SO_4, 6.728; $MgSO_4$, 2.991; $MnSO_4.H_2O$, 0.258; in mg/kg: $ZnCO_3$, 37.6; KI, 0.337; $FeSO_4.7H2O$, 292; $CuSO_4.5H_2O$, 33.2; Na_2SeO_3, 0.33; $Cr(Acetate)_3.H_2O$, 4.78; MoO_3, 1.51; $CoSO_4.7H_2O$, 4.79.

TABLE 2. Schedule and Diet Codes for Antithiamin Studies

PHASE	LENGTH OF PHASE	DIET GROUPS
1. (Quarantine)	1 week	A
2. (Depletion)	14-16 days	A,B
3. (Repletion)	4 weeks	A, C-L

DIET CODE	DIET	THIAMIN LEVEL mg/kg dry weight
A	Semipurified	20.0 (non deficient control group)
B	Semipurified	0 (deficient diet)
C	Semipurified	3.0
D	Semipurified	20.0
Chicken-Containing Diets		
E	Frozen Chicken	3.0
F	Frozen Chicken	20.0
G	Thermally Processed	3.0
H	Thermally Processed	20.0
I	Gamma Irradiated	3.0
J	Gamma Irradiated	20.0
K	Electron Irradiated	3.0
L	Electron Irradiated	20.0

TABLE 3. Proximate Analyses, Calcium, Phosphorus and Thiamin Contents in Chicken Test Meats

Test Meat	Moisture %	Protein %	Fat %	Ash %	Phosphorous %	Calcium %	Thiamin mg/kg Wet Wt.	Thiamin mg/kg Dry Wt.
Frozen								
Mean	64.45	18.74	13.92	1.51	0.248	0.005	0.370	1.040
SD	±.41	±0.54	±.36	±.09	±.014	±.001	±.0456	±0.128
n=8*								
Thermal								
Mean	64.00	18.86	14.34	1.54	0.251	0.004	0.083	0.230
SD	±.27	±0.32	±.15	±.06	±.019	±.001	±0.002	±0.004
n=8*								
Gamma Irradiated								
Mean	63.37	19.49	14.34	1.58	0.237	0.004	0.097	0.264
SD	±.95	±0.56	±.70	±.08	±.018	±.001	±0.009	±0.025
n=8*								
Electron Irradiated								
Mean	65.19	19.03	12.85	1.54	0.241	0.004	0.243	0.698
SD	±.61	±0.51	±.60	±.07	±.021	±.001	±0.046	±0.133
n=8*								

*For thiamin analyses, n=3. The meats were sampled three times and each sample was assayed at three different dilutions.

TABLE 4. Thiamin Contents of Repletion Diets

	Thiamin	
	mg/kg wet weight[1]	mg/kg dry weight[2]
	Mean ± SD[3]	Mean
3.0 mg/kg Diets		
Frozen	1.82 ± 0.19	3.0
Thermal	1.68 ± 0.16	2.8
Gamma Irradiated	1.71 ± 0.18	2.8
Electron Irradiated	1.88 ± 0.06	3.1
Semi-Purified	3.38 ± 0.08	3.4
20.00 mg/kg Diets		
Frozen	11.6 ± 1.9	19.0
Thermal	11.6 ± 2.0	18.8
Gamma Irradiated	12.6 ± 1.3	20.2
Electron Irradiated	14.6 ± 1.3	24.2
Semi-Purified	22.7 ± 2.3	22.7

[1]Microbiological assay

[2]Calculated by estimating the moisture content of each diet on the basis of the original proximate analyses of the test meats (meat diets contained 35% dry weight of the respective test meat).

[3]Two samples were removed from each diet. Each of these was extracted and assayed at three different dilutions. The means and SDs of each set of six values are presented.

TABLE 5. Growth of Thiamin-Deficient Rats Repleted with Semi-Purified or Chicken-Based Diets (Males)

Group	Treatment	Initial Weight (g)[1]	Final Weight (g)[1]	Average Daily Gains(g) Week 1	Week 2	Week 3	Week 4	Overall Average (g)/Day
A	Non-deficient	190.1 ± 13.3	377.5 ± 37.6	8.9	7.3	6.8	4.9	7.0
C	Dry - 3.00	159.6 ± 18.4	350.5 ± 35.0	9.3	6.9	6.4	5.2	6.9
D	Dry - 20.00	159.9 ± 21.2	346.7 ± 33.3	7.8	7.1	6.1	4.9	6.5
E	Frozen - 3.00	160.8 ± 22.4	386.9 ± 38.0	12.4	8.6	7.0	6.1	8.4
F	Frozen - 20.00	159.5 ± 25.5	392.0 ± 51.4	11.4	8.1	7.3	7.4	8.4
G	Thermal - 3.00	158.4 ± 19.3	383.3 ± 31.5	12.1	8.6	7.7	5.3	8.4
H	Thermal - 20.00	159.3 ± 19.6	398.7 ± 25.5	13.4	9.1	8.2	5.5	9.0
I	Gamma - 3.00	160.6 ± 21.4	392.8 ± 20.3	12.4	8.3	8.2	5.7	8.6
J	Gamma - 20.00	159.5 ± 22.9	380.7 ± 37.2	11.7	8.2	7.3	5.5	8.1
K	Electron - 3.00	159.2 ± 18.7	388.5 ± 36.1	11.3	8.5	8.1	5.7	8.4
L	Electron - 20.00	160.0 ± 20.8	375.9 ± 31.9	10.5	8.3	7.3	5.5	7.9

[1] Mean ± S.D.

TABLE 6. Growth of Thiamin-Deficient Rats Repleted With Semi-Purified or Chicken-Based Diets (Females)

Group	Treatment	Initial Weight (g)[1]	Final Weight (g)[1]	Average Daily Gains(g) Week 1	Week 2	Week 3	Week 4	Overall Average (g)/Day
A	Non-deficient	152.5 ± 9.9	239.5 ± 19.0	3.3	4.0	3.5	3.0	3.5
C	Dry - 3.00	143.2 ± 15.1	231.5 ± 28.1	4.4	4.0	2.4	2.7	3.4
D	Dry - 20.00	142.4 ± 13.3	234.6 ± 31.3	4.3	3.8	2.8	3.1	3.5
E	Frozen - 3.00	142.4 ± 13.4	261.2 ± 23.2	6.8	4.3	3.8	3.5	4.5
F	Frozen - 20.00	142.6 ± 15.7	268.2 ± 35.1	7.2	5.3	3.2	3.9	4.9
G	Thermal - 3.00	141.9 ± 13.2	258.1 ± 31.2	6.7	4.7	3.6	3.2	4.5
H	Thermal - 20.00	143.1 ± 13.1	262.0 ± 24.7	6.7	4.7	3.7	3.4	4.6
I	Gamma - 3.00	144.3 ± 12.6	255.3 ± 25.2	6.4	5.1	3.0	3.0	4.3
J	Gamma - 20.00	142.9 ± 12.8	254.8 ± 18.6	6.1	4.3	3.8	2.8	4.2
K	Electron - 3.00	141.3 ± 13.3	261.1 ± 26.5	7.5	5.2	3.4	3.3	4.8
L	Electron - 20.00	143.5 ± 11.8	253.9 ± 21.7	6.0	4.9	2.9	3.3	4.3

[1] Mean ± S.D.

TABLE 7. Mean Daily Weight Gains of Male Thiamin-Deficient Rats During Repletion With Semi-Purified or Chicken-Based Diets (Males)

	Dates of Weighings										
Group	10/26-27	10/27-30	10/30-11/1	11/1-3	11/3-6	11/6-8	11/8-14	11/14-16	11/16-17	11/17-20	11/20-22
	Mean Weight Gain (g)/Day)[1]										
A	8.7	9.0	8.8	4.6*	10.6	4.9	7.2	5.7	-0.8	6.7	5.0
C	10.3	9.8	8.0	3.9**	9.9	5.4	6.5	6.0	-0.9	7.0	5.7
D	11.6	7.0	7.2	4.6	9.8*	5.5	6.3***	5.6	0.2	6.3	5.3
E	21.8	10.6	10.4	5.5	12.3	6.1	6.8*	7.7	-0.9	9.3	4.8
F	21.5	8.6	10.5	4.0	11.8	6.5	7.5**	6.6	0.1	10.8	6.1*
G	19.0	11.4	9.7	5.1**	11.8	7.1	7.9	7.3	-0.7	7.1	5.7
H	21.3	11.4	12.1	5.9	12.0*	8.1	8.3*	8.1	-2.3	8.4	5.0
I	21.3	11.2	9.6	4.6	9.4	10.3	8.6	7.1	-7.3	9.5	6.6
J	22.0	9.4	10.0	3.7*	11.1	8.3	7.6*	6.4	-5.7	9.3	5.4
K	21.9	9.3	8.9	5.3**	10.6	8.5	8.5	6.8	-4.7	8.4	6.9
L	20.4	8.4	8.7	4.9*	11.7	6.7	7.6*	6.5	-5.7	8.2	7.0

[1] Time intervals between weighings varied from 1 to 6 days. Weekly averages for Table 5 were calculated for the following periods: Week 1 (10/26-11/1), Week 2 (11/1-11/8) Week 3 (11/8-11/16), Week 4 (11/16-11/22).

*Indicates loss of one (*), two (**) or three (***) animals after cardiac puncture.

TABLE 8. Mean Daily Weight Gains of Female Thiamin-Deficient Rats During Repletion With Semi-Purified or Chicken-Based Diets (Females)

	Dates of Weighings									
Group	11/1-3	11/3-6	11/6-7	11/7-14	11/14-16	11/16-17	11/17-20	11/20-22	11/22-24	11/24-27
	Mean Weight Gain (g)/Day[1]									
A	2.2	4.4	2.2	4.0	1.9	1.3	4.1	2.3	3.8	3.0
C	4.4	5.3	1.8	4.0	1.3	0.7	3.8	2.6	2.7	2.8
D	4.5	4.8	2.2	3.8*	2.4	2.8	3.1	3.6	3.3	2.7
E	9.5	6.3	3.1	4.3	2.9	0.6	5.5	4.4	2.4	3.6
F	11.4	6.5	1.2	5.3	-0.4*	5.7	4.8	4.2	4.5	3.4
G	9.9	5.8	3.3	4.7*	1.9	3.5	4.7	3.2	4.0	2.6
H	9.4	6.4	2.2	4.7	2.2	2.3	5.1	3.3	3.3	3.5
I	8.7	6.5	1.3	5.1	0.9**	1.4	5.0	4.2	3.2	2.1
J	8.9	5.6	2.2	4.3*	3.5	0.9	5.0	2.7	5.3	1.3
K	9.8	7.0	4.5	5.2**	1.5	0.1	5.7*	1.3	7.1	2.1
L	8.0	6.4	1.1	4.9**	3.0*	-1.0	4.2	3.4	4.1	2.7

[1]Time interval between weighings varied from 1 to 7 days. Weekly averages for Table 6 were calculated for the following periods: Week 1 (11/1-11/7), Week 2 (11/7-11/14), Week 3 (11/14-11/20), Week 4 (11/20-11/27).

*Indicates loss of one (*) or two (**) animals after cardiac puncture.

TABLE 9. Summary of Erythrocyte Transketolase Activity in Thiamin-Deficient Rats During Repletion With Semi-Purified or Chicken-Based Diets[1]

Day of Repletion	Semi-Purified	Frozen	Thermal	Gamma	Electron
		Males - 3 mg thiamin/kg			
7	0.17 ± .04	0.17 ± .02	0.16 ± .03	0.17 ± .03	0.17 ± .03
14	0.23 ± .03	0.20 ± .02	0.20 ± .03	0.20 ± .02	0.20 ± .02
27	0.21 ± .04	0.19 ± .02	0.20 ± .02	0.18 ± .02	0.20 ± .01
		Males - 20 mg thiamin/kg			
7	0.20 ± .04	0.19 ± .03	0.19 ± .02	0.21 ± .03	0.24 ± .06
14	0.25 ± .03	0.24 ± .02	0.26 ± .04	0.27 ± .03	0.26 ± .05
27	0.25 ± .03	0.26 ± .03	0.26 ± .03	0.25 ± .03	0.26 ± .04
		Females - 3 mg thiamin/kg			
7	0.15 ± .04	0.15 ± .02	0.16 ± .01	0.15 ± .02	0.16 ± .02
14	0.18 ± .02	0.20 ± .03	0.21 ± .02	0.20 ± .05	0.19 ± .02
27	0.22 ± .03	0.21 ± .04	0.24 ± .04	0.23 ± .04	0.23 ± .03
		Females - 20 mg thiamin/kg			
7	0.17 ± .03	0.18 ± .02	0.19 ± .03	0.18 ± .02	0.18 ± .02
14	0.21 ± .02	0.22 ± .03	0.22 ± .03	0.21 ± .03	0.21 ± .04
27	0.24 ± .03	0.25 ± .02	0.26 ± .04	0.25 ± .04	0.25 ± .06

[1] IU/ml red cells; mean ± S.D.

TABLE 10. Summary of Thiamin Pyrophosphate Stimulation (TPP Effect)*

GROUP	MALES			FEMALES		
	DAY 0			DAY 0		
A Mean	5.47			1.56		
S.D.	4.59			3.46		
B Mean	16.79			1.39		
S.D.	17.47			4.81		
	Day 7	Day 14	Day 27	Day 7	Day 14	Day 27
A Mean	1.76	5.53	5.88	2.26	4.85	2.45
S.D.	3.17	2.03	4.22	3.58	0.84	4.06
C Mean	1.89	2.17	5.74	4.01	3.86	2.39
S.D.	3.44	2.84	2.22	4.62	4.05	4.18
D Mean	3.20	5.85	3.95	3.51	4.83	4.80
S.D.	3.72	3.78	3.60	4.37	3.17	4.08
E Mean	2.64	7.35	4.84	6.13	4.31	5.68
S.D.	3.99	3.92	3.15	5.48	5.88	3.92
F Mean	1.26	5.35	5.36	3.79	3.00	4.68
S.D.	2.65	3.31	2.99	4.71	3.16	3.52
G Mean	6.57	5.28	5.17	1.52	4.22	2.80
S.D.	5.98	4.06	2.79	3.37	4.53	3.28
H Mean	1.35	4.85	3.18	2.54	3.71	5.33
S.D.	3.00	3.01	2.79	4.95	3.37	3.14
I Mean	4.15	3.68	5.06	3.58	2.29	3.89
S.D.	4.43	3.95	4.73	5.24	4.34	4.57
J Mean	1.37	5.53	4.73	2.52	3.06	2.56
S.D.	2.48	3.35	2.75	3.52	3.54	2.97
K Mean	4.37	7.95	4.51	2.30	3.80	3.89
S.D.	6.03	4.83	4.29	3.98	4.02	5.38
L Mean	1.51	5.34	4.55	8.14	3.17	3.43
S.D.	2.69	4.66	3.95	6.25	3.44	4.28

*All stimulation values are expressed as percent.

TABLE 11. Analysis of Variance Significance levels[1]

	Parameters[2]	Food	Vitamin	Interaction
Males				
Day 7	ETK	0.03	0.00	0.40
	ETK Stim	0.05	0.00	0.43
	TPP Effect	0.54	0.00	0.10
Day 14	ETK	0.37	0.00	0.08
	ETK Stim	0.50	0.00	0.16
	TPP Effect	0.15	0.92	0.04
Day 27	ETK	0.43	0.00	0.55
	ETK Stim	0.52	0.00	0.38
	TPP Effect	0.94	0.30	0.68
Females				
Day 7	ETK	0.35	0.00	0.90
	ETK Stim	0.52	0.00	0.98
	TPP Effect	0.14	0.56	0.05
Day 14	ETK	0.02	0.00	0.64
	ETK Stim	0.05	0.00	0.64
	TPP Effect	0.60	0.83	0.85
Day 27	ETK	0.32	0.01	0.94
	ETK Stim	0.32	0.00	0.89
	TPP Effect	0.49	0.63	0.31

[1] P values obtained by ANOVA on five food groups, two vitamin levels. (Groups C through L).

[2] ETK, Erythrocyte transketolase activity.
ETK-Stimulated, ETK activity in the presence of added thiamin pyrophosphate co-factor.
TPP Effect, % increase in ETK due to added TPP.

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